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FINAL REPORT

HIGH RESOLUTION DETECTION

OF RADIATION

By

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INTRODUCTION

The objectives of our project have been

1. Creation of new systems of detecting radiation in which there are incorporated such properties as:

    a. very high spatial resolution

    b. low sensitivity to non-ionizing radiation

    c. low inherent background

    d. high sensitivity to ionizing radiation

2. Creation of new devices to facilitate detection of radiation with adequate spatial resolution but with less manipulation necessary than is the case with existing methods.

As a result of objective (1), came the development of wet process autoradiography and later the perfection of protective layer techniques for use with the commercially available stripping films. Wet process autoradiography has been widely adopted[1] for solution of special radiation detection problems.

The beta ray microscope came as a result of work on objective (2). A bibliography of articles and reports to which workers on this project contributed is given in Appendix I.

In this report are descriptions of additional new processes which have been used successfully in high spatial resolution detection of radiation.

It is our belief that the techniques now available provide adequate support for research work in which precise location of radioactive tracers is needed. This was not true at the time the project was started. It is therefore our conclusion that our efforts should be used in other areas where more significant problems exist. For us, these are primarily in the study of fundamental mechanisms in the interaction of radiation and matter.

In this final report, we are presenting the results of experiments in which new types of films, suitable for high resolution autoradiography were produced. In one group, the basic silver halide system was retained, but incorporated into films in such a way as to maximize resolution despite the presence of a visible grain structure. In others, molecular reaction systems were used so as to eliminate the grain structure problem. Some illustrative applications of the successfully developed systems are shown.

Finally, some of our unsuccessful attempts at new film development are discussed.

RESEARCH RESULTS

I. HIGH DENSITY, THIN SILVER HALIDE FILMS

The technique described below was the final development of an effort to make a very thin (1 micron) but dense film of silver halide grains. The principles of action of such a film are well known but the technique for manufacture had to be created.

The film produced is considered quite satisfactory for application to problems involving small specimen areas (1 square centimeter or less.)

Method For Making Silver Bromide Thin Film

A. Ag-KOH Solution.

1. Make up 1% silver nitrate in distilled water.

2. Add a few drops of dilute ammonium hydroxide. The solution will turn dark brown.

3. Add more ammonium hydroxide very slowly until the brown precipitate is almost gone.

4. Make up a 1% KOH solution and add to the above mixture in a 1:2 proportion. (If using 100 ml. $AgNO_3$ solution, add 50 ml. KOH solution). The mixture will again precipitate.

5. Add a few more drops very slowly of dilute NH_4OH, but do not let the solution become

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entirely clear. If the solution should clear,
then add more AgNO$_3$ until a permanent precipitate
forms.

B. Reducing Solution.

This solution should be prepared one week prior
to using.

700 ml. distilled water, 80 gm. sugar

When dissolved add:

175 ml. ethanol, 3 ml. nitric acid

Water to make 1000 ml.

Use 1/25 as much reducing solution as Ag-KOH
solution. The reducing solution is poured into
the Ag-KOH solution and quickly stirred and
poured into contact with substance to be silvered.

Method For Making Plastic Film

The plastic film is made for a 2% solution of parlodion
in amyl acetate. The film is formed by placing a drop of
the 2% parlodion in a large watch glass containing distilled
water. The plastic spreads over the surface of the water
and a thin film is formed.

When the film has formed, take one part of the reducing
solution and pour into a beaker containing 25 parts of the
Ag-KOH solution and stir quickly. As soon as this is well
mixed, quickly decant the distilled water out from under the
plastic film and replace it with the silvering solution.
This is easily accomplished by pouring the silvering solution

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towards the edge of the film -- the solution will flow under
the film.

As this is allowed to stand, the solution underneath
the film will turn first black, then dark brown, then a
golden grey color.

When the film is uniformly coated with silver, decant
the silvering solution out from under the film and replace
with several changes of distilled water until the distilled
water remains clear.

The silver grains are now on the water side of the
floating film. The film may now be picked up on whatever
carrier one wishes (slides, etc.) merely by placing the
carrier over the film and then quickly inverting so the
film is picked up grain side up. It is necessary that the
grains be in this position as the processing solutions
needed later will not penetrate the plastic support. Also,
in this way, the plastic support acts as a protective layer
between the sample and the film, thus in some measure helping
to prevent chemical reaction between the film and the object
to be autoradiographed.

After the film has dried well -- a hot air blower may
be used to hasten this -- the silver is ready to be converted
to silver bromide. All steps from here on must be carried
out in a hood with red light illumination only (wratten filter
#2 at two or three feet distance from the film is satisfactory).
The silver grains are converted to silver bromide grains
merely by placing the film in a container holding a few

drops of liquid bromide. Here one must experiment until
the correct amount of bromine is used to fill the container
with fumes just sufficient to convert the silver to silver
bromide, without giving an overdose of bromine and burning
the plastic. When this procedure has been performed correctly,
the film will turn an even white color and will still be
transparent. The yellow color of the bromide is just barely
perceptible.

After the film has had sufficient exposure to the
bromine it is removed from the bromine and aired in the hood
to get rid of any excess free bromine, and then the film
is coated with gelatin by dipping the film in a 5% gelatin
solution, draining, and allowing the film to dry slowly in
the air.

The gelatin is necessary, among other things, to help
hold the grains in place during processing, as well as
acting as a sensitizer on the silver bromide grains.

Discussion

A. Film Formation.

Forming the film directly on the silvering solution
instead of on water first, was tried. It was found that a
smoother film was achieved in this manner, due mainly to
less handling. However a large smooth film is not a
necessity as the area of film needed for most specimens
is small. Also, forming the plastic film directly over
the silvering solution gave a much heavier silver deposit,

in fact too heavy a deposit, while at the same time some of
the silver grains were deposited within the plastic during
its formation, and these grains could not be reached by
the processing solutions.

On the other hand, forming the film first on distilled
water and then replacing the distilled water with silvering
solution resulted in films with a thinner layer of silver
extremely tightly packed and the grains on this film were
completely cleared by fixing solutions indicating no grains
could be deposited within the plastic itself, but only on
the surface of the plastic. And finally, film formed in
this manner halogenated more rapidly, uniformly and completely.

B. Film Processing.

Film development was tried with various commercial
developers as well as with the physical development technique.
All methods of development worked with the most satisfactory
being D-19 used undilute for 20 seconds. Fixation was
carried out with F-6 for one minute. Actually the film
clears grossly in four or five seconds. Following fixation
the film is rinsed in several change of water and dried.

C. Film Sensitization.

Film sensitization experiments were carried out testing
the effect of:

> 1. Combined halogenation -- specifically bromine
> and iodine. This was carried out by briefly
> shortening the film's exposure to bromine and

following this exposure with an exposure to iodine
fumes. Although various combinations in timing
were tried no conclusive evidence was obtained
to indicate this method to be either beneficial
or detrimental to the process

2. Different types of gelatin were tried and here
again the results were not consistent enough to
indicate any increase in sensitivity. However,
the use of Kodak pure gelatin gave much cleaner
results. Although all gelatins were filtered before
use, some of them showed minute fragments which were
not present in the Kodak gelatin.

3. Experiments were also carried out to determine
if the use of NH_4OH would act as a sensitizer to
the film. Again the evidence accumulated was not
conclusive one way or another.

4. The fourth method tested was the additon of a
known sensitizer to the gelatin. This was accomplished
by adding the sensitizer to the melted gelatin before
the film was dipped. Results of tests run with
this sensitizer indicated an increase in sensitivity
of the film.
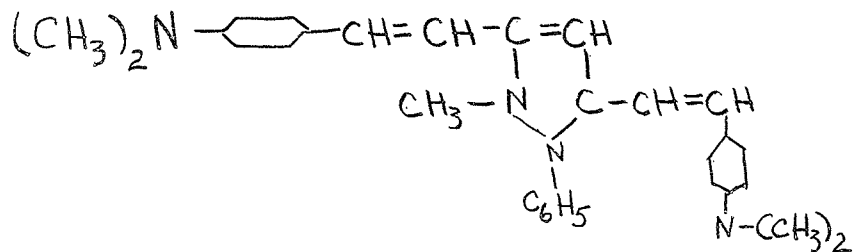
5. The effect of temperature on the film during its exposure time was tested and this had no effect on sensitivity. However, when temperatures above 25° C. were used, the gelatin became too brittle and did not process well, if at all.

These experiments were all run using radioactive chlorella as a test source. The chlorella was incorporated in the gelatin coating used merely by adding a measured amount to the gelatin before dipping the film in the gelatin. Inactive chlorella was used as a control. Very good results were obtained using this as a test organism, quite consistent results being obtainable. Test results are shown in Fig. 1.

The film was also tested on radioactive metals, and x-ray. Good response was obtained to thallium and to x-rays. Experiments run with radioactive nickel would not give consistent results, although some good autoradiographs were obtained, they could not always be duplicated. Much difficulty was encountered trying to prevent overexposure of the metal to the bromine, although heavy (5μ) plastic protective layers of vyns were used between the metal and the film, the bromine would still penetrate the film and etch the metal.

The film was also tried on rat thyroid tissue. Satisfactory results were not obtained as a system had to be worked out to keep the tissue from drying out while under

the film, as water soluble plastic washed out from under the film during photographic processing, and plastics which were not water soluble fogged during photographic processing. Obviously some entirely different system for maintaining the tissue integrity would have to be developed before readable autoradiographs can be obtained using this silver bromide film.

The thickness of this silver bromine film was determined by beta absorption and results indicated that the film is 1.4 microns thick.
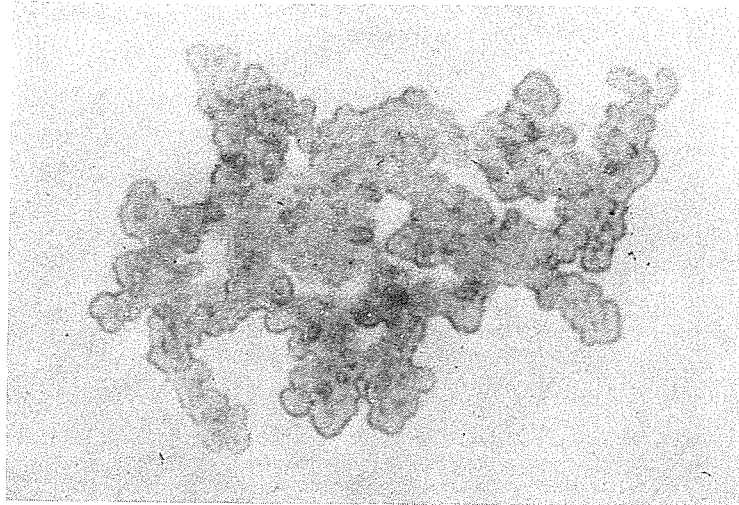
(a)
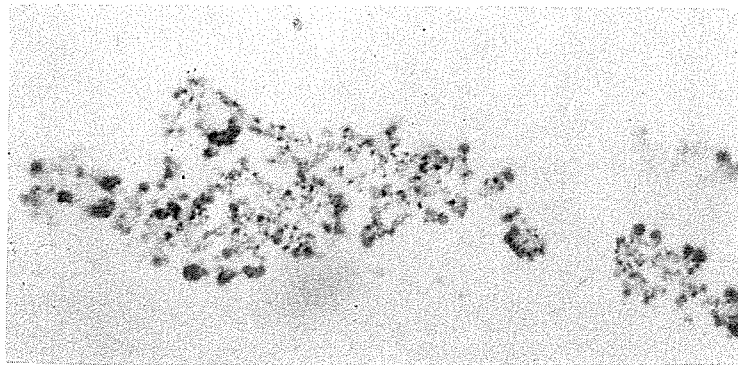
(b)

(c)

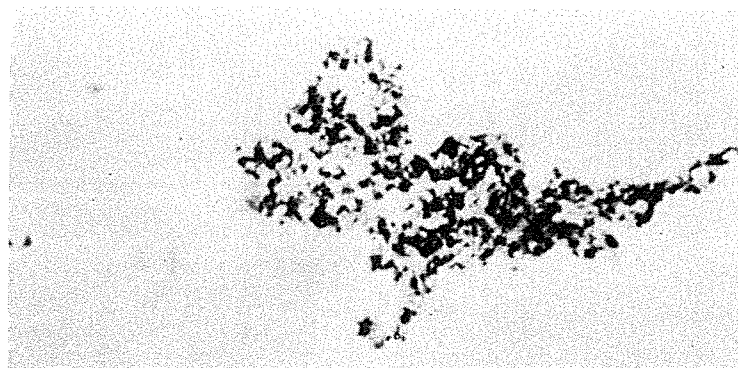Fig. 1: Autoradiographs of Chlorella tagged with C^{14}: (a) is a 1000 x microscopic enlargement of inactive Chlorella used as a control; (b) and (c) are 1000 x microscopic enlargements of autoradiographs of Chlorella tagged with C^{14}. Exposure times were about 24 days.

II. MOLECULAR "DYE FORMING" FILMS[*]

The important characteristics of a detecting layer
which determines resolution in autoradiography and micro-
radiography are the following:

a. thickness of film;

b. sensitivity of the detecting layer to ionizing
    radiation;

c. density of packing of the sensitive material;

d. particle size of the sensitive system.

Up to the present time, silver halide systems have
yielded the best results. Much excellent work has been done
to optimize the above mentioned characteristics. But a
major limitation of any silver system is the particle size.
In order to impart photosensitivity to a silver emulsion,
some kind of crystalline structure is necessary. When
enlargement of the order of 1000 x or greater is desired,
there will be a loss in resolution due to the finite size
of the silver grains.

In order to optimize the characteristics listed in a,
c, and d, a film was prepared in which a dye molecule rather
than a reduced silver grain was produced upon exposure to
ionizing radiation. The sensitive system is molecular rather
than crystalline in size. Furthermore since the system is
a solid solution rather than a suspension, the film has no
insensitive regions.

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[*] See Reference 17, Appendix I

While a, c, and d have been optimized, d) the sensitivity
of the dye films is lower than the silver halide emulsions.

Because of the low sensitivity of the films produced to
date when compared with sensitized autoradiographic emulsions,
the major application of this system will be in microradiography
and possibly dosimetry. In microradiography becasue of
extreme resolution requirements, even the silver halide
films used are usually of low sensitivity. Exposure time
for the dye to achieve a satisfactory image is comparable
to the exposure time required for the fine grained silver
halide films usually used. One thousand diameter and greater
enlargements of the radiographs using dye forming films
can be made directly without resolution loss due to particle
size. Both x-rays and electron beams have been used to
produce microradiographs on these films.

Development Of Dye Forming Films

A. Films Containing Halides And Dye Forming Reagents.

1. Interaction Process

The use of dye forming reagents and halides for
radiation sensitive films was suggested by the work of Mr.
J. Garsou who is studying the radiochemistry of such systems
on another project at our laboratories[2]. Ionizing radiation
tends to cause the release of free halogens from certain
halides. If a dye forming reagent is added to the system,
the free halogen produced upon irradiation will interact
with the reagent to form a dye molecule. The reagent is

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transparent in solution and turns its characteristic color
upon oxidation.

2.   Choice of Halide

Both metallic and organic halides were tried
and P-Phenylenediamine (PPD) was used as the dye forming
reagent.   Either solutions or suspension of the halide and
(PPD) in amyl acetate were made, and then irradiated using
x-ray diffraction unit at 30 PKV and 15 ma.   Metallic bromides
and iodides and also organic iodides were investigated.   The
metallic halides were found to be completely unsatisfactory
because of either strong dark reactions or because of extremely
low yield after rather large doses.   The yield could be
measured roughly by the discoloration produced.   Iodoform
was found to be the most satisfactory of the organic halides.

3.   Choice of Dye Forming Reagent

Polystyrene was used as the plastic to contain
the radiation sensitive system.   The next problem was to
find the best dye forming reagent to use for image formation.
Solutions of polystyrene, iodoform, amyl acetate, and various
dye forming reagents, such as p-phenylenediamine, crystal
violet lactone, benzoyl leuco methylene, and p p' p" methylidy-
nitris (MDT) were used.   Films were then allowed to dry.
These films were then exposed for a given time to a collimated
x-ray beam from an x-ray diffraction unit operating at 30
PKV and 15 ma.   MDT was found to yield the best image in
terms of optical opacity.

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4. Proborling of Constituents Used and Film
Preparation Precaution

Up to this point the constituents which produce
the most satisfactory system were the following:

a. organic halide -- iodoform   0.6   gm

b. dye forming reagent --
    p p' p" methylidynitris (MDT)   0.04 gm

c. solvent -- amyl acetate   20   gm

d. plastic -- polystyrene   1   gm

Other polymers and solvents, such as vinyl
acetate in absolute methyl alcohol were used and found
satisfactory.

The following precautions must be taken in pre-
paring the films. These precautions hold for the films to
be discussed later.

a. The solvents must be relatively dry because
of the instability of the organic halide and
dye forming reagent due to the presence of
moisture.

b. Impurities in either polymers or monomers,
and solvents must not be of such a nature
as to interact with either the organic
halide and dye forming reagent.

c. The films should be dried in a relatively
dry atmosphere, because of the instability
of the organic halide and dye forming reagent
to moisture.

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d. The drying should also be done in a dust
free atmosphere because dust particles in-
corporated into the emulsion interfere with
image detection.

e. The film must be completely dried so that
very little solvent be left in the film.
The films will tend to crack if they are not
completely dried.

Films described above were prepared. Micro-
radiographs using both soft x-rays and collimated electron
beams were produced as follows. A 200 copper wire mesh (an
electron microscope screen) was placed in intimate contact
with the film. The film is exposed to either an x-ray
beam from an x-ray diffraction unit or an electron beam from
an electron diffraction unit. The enlargements in Fig. 2
are made from a microradiograph produced by using an electron
beam. Direct microscopic enelargements were made at 50 x,
124 x and 200 x. The fourth photograph is a 4 x enlargement
of the 200 x microscopic enlargement. The imperfections
in the enlargements are due primarily to mechanical breaks
and scratches in the polystyrene film, to the presence of
undissolved organic halides and dye forming reagent, and
to dust particles incorporated into the film during the
drying process. Microscopic observations at 970 x have
also been made, and no perceptible granular structure has
been observed. The darkening in the background can be

attributed to the sensitivity of the film to light. Therefore, during the optical enlargement process the film tends to darken.

5. Film Instability

It was at this point that the instability under light of this system was observed. The image tended to be lost in background after sustained observation under a microscope. The film was sensitive to light in the optical region, to moisture, and to heat.

Sensitivity to radiation in the visible part of the spectrum was now considered. A spectrophotometer was used as a source of monochromatic light. The film was found to be sensitive to light in the ultraviolet, blue, and green regions while it was relatively insensitive to light in the yellow and red regions.

It is known that free iodine is released from iodoform under exposure to light, heat, and moisture, and therefore, it was believed that if the iodoform could be eliminated after exposure, the film would be more stable.

B. Double Emulsion Films.

In order to find a method of eliminating the iodoform after exposure, a double emulsion system was investigated. MDT was included in a plastic polystyrene film, and iodoform was included in a vinyl acetate film. After casting and drying the polystyrene film containing MDT, the vinyl acetate containing the iodoform film was cast on

top of the polystyrene film. Vinyl acetate is soluble in absolute methyl alcohol while polystyrene is relatively insoluble in absolute methyl alcohol. Thus the film containing the iodoform could be removed after exposure by immersion in absolute methyl alcohol. Such films were prepared, exposures made, and images were formed in the emulsion containing MDT.

The sensitivity was much lower than the single layer films. It was then decided to try to increase the sensitivity by increasing the concentration of MDT in the polystyrene layer. The sensitivity increased, but this system was not too satisfactory because of the extra steps in handling and because of the necessity of increasing the emulsion thickness.

C. Dye Forming Film With Dye Forming Reagent Alone.

1. Film Sensitivity, Constituents and Preparation

Previously, the sensitivity of the films containing rather low concentrations of MDT without any organic halide were tested and the films seemed relatively insensitive to both x-rays and electrons. Now it was decided to try the films with MDT alone but with an increased concentration of MDT. The films were found to be sensitive to both x-rays and electrons, although the sensitivity with respect to the first described system is somewhat lower. This system is much more stable than the system containing iodoform. These films are almost completely insensitive to light in the optical region as shown in tests using a spectrophotometer as an irradiation source.

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The following system using MDT is the most satis-
factory system developed up to the present time.

      a.   dye forming reagent -- MDT   .1 gm

      b.   solvent -- amyl acetate   10   gm

      c.   plastic -- polystyrene   2   gm

The thickness of the emulsions will depend on the amount and
nature of the solvent and plastic, and on the method of
drying. The film preparation is the same as described in (B).

    2. Alternate Preparation Technique

      Finally, an alternate film preparation technique
has been investigated. This method eliminates the necessity
of drying out the solvent, and allows control of the im-
purities added to the system. In this technique the MDT
is added to the monomer. The monomer is polymerized using
either heat or catalytic agents. A solid solution of MDT in
a plastic block is then obtained. This technique has been
tried and found successful with foaming urathane plastics.
This plastic is not transparent, so that styrene and methyl
methacrylate monomer were tried. The following difficulties
were encountered with these monomers.

      A strong "dark reaction" occurred due to the
concentration of reagent, oxidation by catalyst, and oxidation
due to heat. Most catalytic agents contain peroxides which
tend to oxidize the dye forming reagent. One catalyst,
bis-azo-isobutyralnitrite, does not form peroxides and thus
will not oxidize the system upon polymerization. If this

catalyst can be obtained, this may prove to be the proper agent for polymerization.

Summary Of Results And Applications

A. Results.

Enlargements of microradiographs of copper electron diffraction screens are shown in Fig. 2. These enlargements were made from microradiographs using the film prepared with the organic halide. Microradiographs have also been made with the films containing MDT alone yielding similar results. This latter film shows greater stability to heat, moisture and light. The exposure times required to produce detectable images using collimated electron beams are from 30 minutes to one hour. Exposure times to produce detectable images using x-ray beams from an x-ray diffraction unit, copper target, 30 PKV, 15 ma at about 6 inches from the target are also about 30 minutes to one hour. These exposure times are about of the same order of magnitude that is required for the extremely fine grained silver halide films, such as the 548 spectroscopic films, which are usually used in microradiography.

The films using MDT alone have only recently been developed. Therefore, time has not permitted us to investigate quantitatively the sensitivity of the films. Tests were carried out for the films using both iodoform and MDT. Because of the instability of the system, the results of this investigation were rather meaningless. The optical opacity

produced in the film by a given irradiation is used as a measure of sensitivity. Knowing the radiation dose for a given irradiation, the film sensitivity as a function of dose can be found by measuring the optical opacity using a spectrophotometer for the various irradiations. The films containing iodoform are sensitive to the light used in measuring the optical opacity, thus the optical opacity of the films changed during these measurements. So the original attempt to measure the film sensitivity was perturbed by the method of detection. These measurements should be able to be performed with the new film containing the dye forming reagent alone. Both the energy dependence and dose dependence should be investigated.

B. Application To Dosimetry And Irradiation Pattern Studies.

An application of these radiation sensitive layers is their use as dosimeters. Investigation could be carried out to measure the film opacity as a function of dose. If a linear relation holds between optical opacity and dose and if the reproducibility of the system is good enough, this application could be directly made.

There is also a possibility of making large plastic blocks containing MDT in solid solution with which dose patterns could be measured. Upon irradiation these blocks will change color outlining the irradiation pattern in the plastic block. Such blocks have been made, but a rather large

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"dark reaction" has occurred in their production. Still, irradiation patterns have been observed in the blocks after exposure to collimated x-ray beams. These patterns are not too easily observed, of course, because of the background but it is believed that this "dark reaction" can be greatly reduced, so that its effect will be negligible.
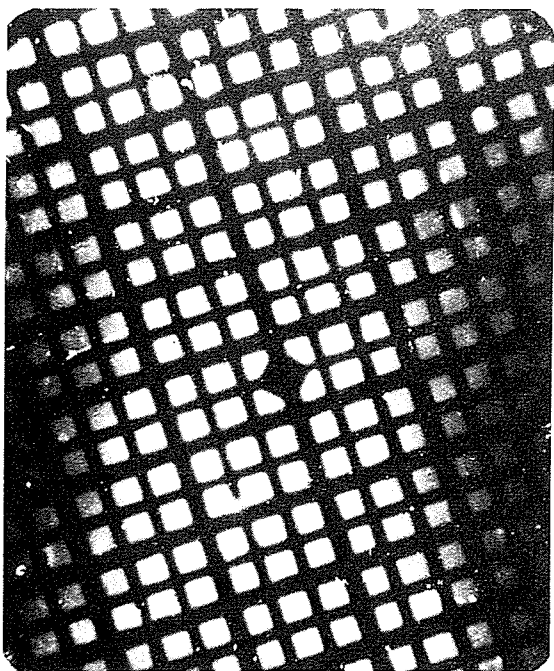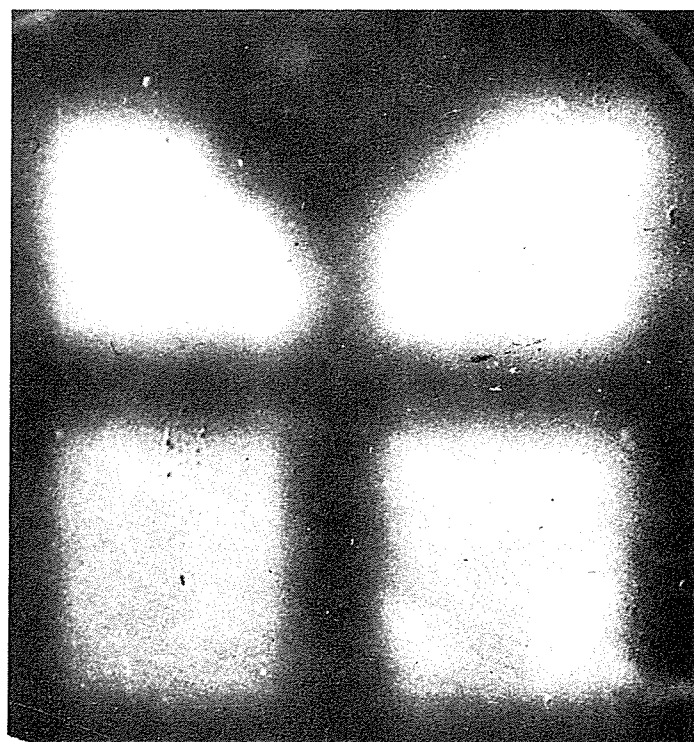
50 x
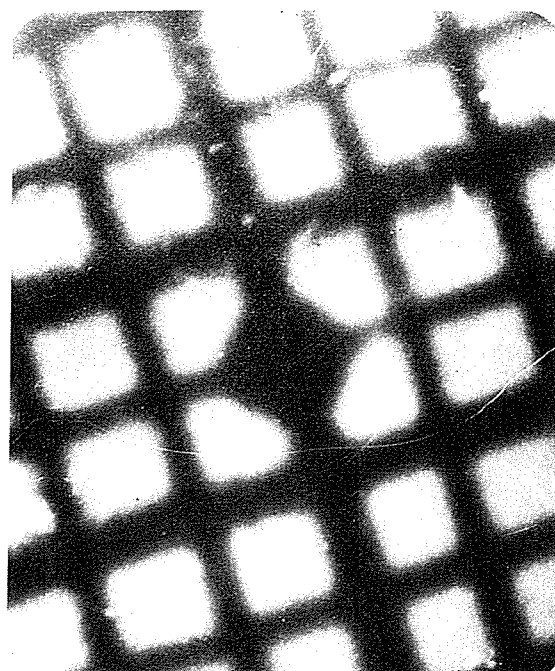
124 x

200 x

800 x

Fig. 2: Use of molecular "Dye Forming" films for recording patterns created by electrons. Shown are 50 x, 124 x, 200 x, and 800 x optical projection enlargements made with microscope. Image is made with 200 mesh electron microscope screen resting on Dye Forming film illuminated with collimated electron beam. No grain structure in original image is apparent.

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III. NEW TECHNIQUES TESTED WITH INDEFINITE OR NEGATIVE
    RESULTS

In any exploratory program such as this one, there are
many experiments which end in failure. For those with
interest in this field, the following are presented so that
they may avoid our errors and failures.

A. Monomolecular Silver Halide Film Experiments.

In an attempt to develop a sensitive silver halide
material fulfilling the requirements essential to high-
resolution autoradiography, of

1. extreme thinness

2. dense packing of halide grains

efforts were made to produce true monomolecular layers or
"monolayers" of silver halide (the word monolayer is here
used in its strict sense, meaning one molecule thick, rather
than in the sense of one grain thick, as in some previous
reports of this project). The production of these layers
has been studied for many years and the technique has been
described adequately in the literature (see, for example,
Blodgett[3] and Sobotka[4]). The principle of the method
is to deposit a minute quantity of a fatty acid or other
polar substance on a water surface. The water attracts
the so-called hydrophilic-COOH groups of the fatty acid
molecules strongly enough to prevent their escape from the
water surface -- and consequent evaporation of the film --
but not strongly enough to cause dissolution of the film in
the water substrate. In these circumstances the film

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resembles a two-dimensional gas but if lateral pressure is applied to it by suitable barriers, the intermolecular distance can be made less than the range of the inter-molecular forces and the layer of molecules then behaves as a two-dimensional liquid which resists compression strongly. The compressed film can be withdrawn from the water surface onto a suitable metal plate, and the mounted film then con-sists of a true monolayer with the smallest thickness and densest packing possible.

Metallic ions can be incorporated into these films by floating the fatty acid on a substrate consisting of water adulterated with a salt of the desired metal. If the pH of the substrate is properly adjusted, the hydrophilic heads of the fatty acid molecules, protruding a little below the water surface, combine with free cations which remain attached to the acid molecules during subsequent withdrawal of the film from the liquid surface. In this way a monolayer consisting of a metallic salt of a fatty acid may be built up. Chemical treatment of the monolayer can be undertaken to a limited extent and in certain cir-cumstances the long-chain group can be replaced by halogen or other atoms.

In the present experiments, it was proposed to form a film of stearic acid on a substrate containing silver ion. The resulting film of silver stearate molecules would, after removal on a chromed plate, as described by Sobotka[4]

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and others, be converted to silver halide by treatment with chemical solutions or by direct exposure to halide vapor.

The difficulty of this scheme is that some kind of crystalline structure is necessary to impart photographic sensitivity to the layer. However, by depositing a "multi-layer" of twenty or thirty superimposed layers, a film with a regular structure can be made, and it is reasonable to assume that it would exhibit photographic sensitivity. This view is supported by the work of Bikermann[5], Astbury et al[6], and Stenhagen[7], which attributes a quasi-crystalline structure to multi-layers of various kinds, and by Fankuchen[8] and Bikermann and Schulman[9], who observed x-ray diffraction patterns compatible with the existence of micro-crystals in the layers.

The films were made by dissolving some stearic acid in a tiny drop of benzene and depositing it in the center of a loop of waxed thread floating on the liquid surface. Compression of the film which remained after evaporation of the benzene was achieved by depositing another benzene drop, containing oleic acid, in the space between the thread and the walls of the container (Fig. 3). The oleic acid acts as a "piston oil" because of its higher surface pressure, and compresses the loop and its contained film until the stearate layer assumes its condensed form. The film is removed from the substrate by inserting a small chrome plated plate vertically in the liquid and withdrawing it slowly. The

Metal plate on
which film is
removed

thread

oleic acid
here

stearic acid
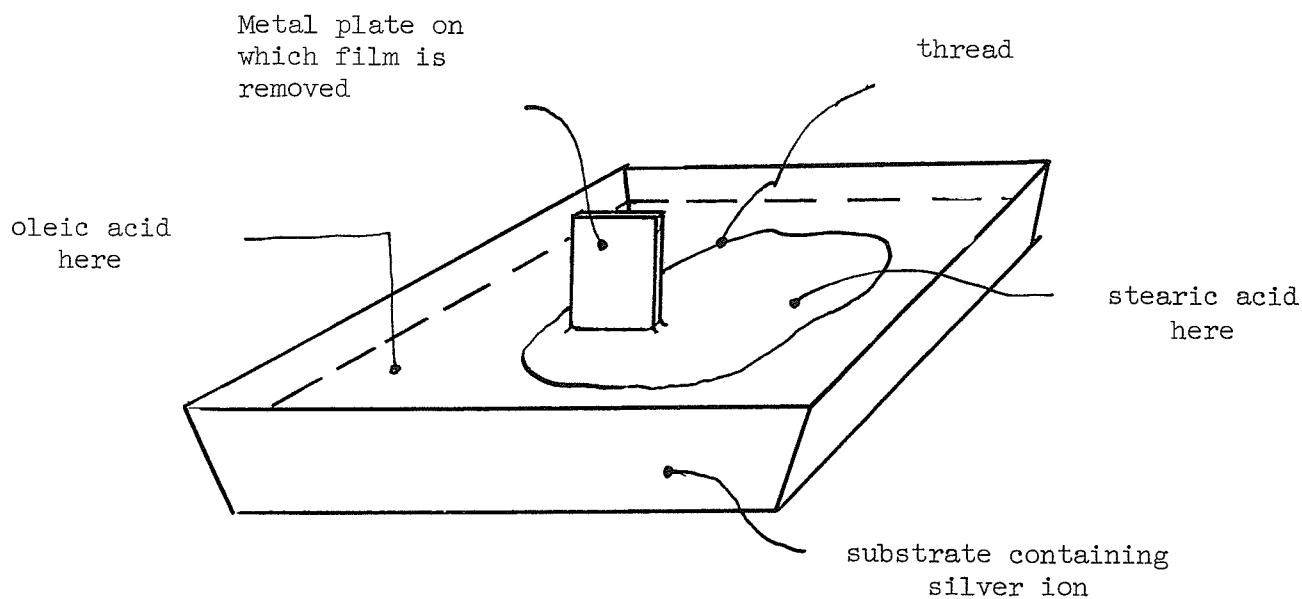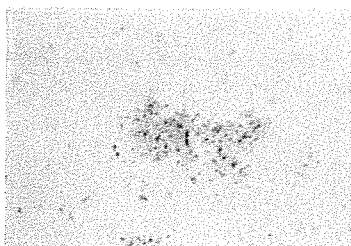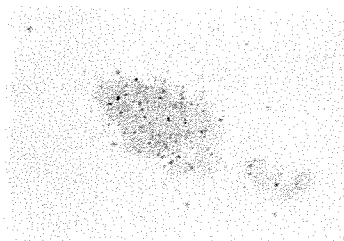here

substrate containing
silver ion

Fig. 3: Arrangement for forming monolayers.

(a)

(b)

Fig. 4: Showing apparent improvement in resolution when the
gelatin concentration in the autoradiographic emul-
sion is reduced by dissolving. Undissolved emulsion
at (a), dissolved at (b). The test-object is C-14
labelled Chlorella.

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film attaches itself to the plate and any number of layers
can be placed on top of each other by moving the plate up
and down slowly. The progress of the operation can be followed
by noting the diminution in area of the waxed loop.

The water was 0.001 N in $AgNO_3$ and was buffered at
a pH of 8.0. The silver stearate layer was successfully
withdrawn and was converted to halide by exposure to the
vapors of iodine and bromine. After exposure to light the
films were developed and fixed with D-19 and F-6, but without
any blackening being apparent under the microscope. A
remarkable feature was the extreme resistance of the films
to these manipulations, in sharp distinction to commercial
emulsions which are sometimes severely damaged in processing.
This great strength is not gained at the expense of permanent
attachment to the mounting plate, for removal is easily effected
by lowering the plate gently into dilute H_2SO_4, when the film
floats off the plate. If such removal were not possible, it
is difficult to envisage any large scale application to auto-
radiography, since the advantage of extreme thinness would be
offset by the difficulty of achieving intimate contact between
a biological specimen and a plate-mounted emulsion.

Failure to observe response to light could be due
to:

1. Absence of crystalline structure in the layers;
2. Presence of ion other than Ag^+ in the substrate.
    This is known to have a profound effect on the
    nature of the resultant film.

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3. Insufficient attention to cleanliness. Many of the precautions (triply-distilled water, stearate-coated dishes, etc.) advised by the standard texts, were not observed -- and indeed, would render routine preparation of the films in the dark an exacting process. The possible effects on the character of the films were not investigated.

Though the preliminary experiments met with no success, the method should be investigated further as it offers the utlimate in thinness and dense packing. The points which are uncertain are the existence of sufficient crystalline structure to impart sensitivity, and the possibility of catastrophic spread of whatever photographic effects occur through an essentially continuous layer of halide. The handling properties of the films are a very attractive feature.

B. Diastase Experiments.

Though it is a relatively simple matter to obtain, by the techniques developed in this project, a silver halide layer of almost vanishing thickness, it is considerably more difficult to arrange a sufficiently dense packing of grains within the layer. Experiments carried out by Comer and Skipper[10] suggested the following approach.

Eastman Kodak Permeable-Base Stripping Film (experimental) with a sensitive layer of 5μ thick was mounted

emulsion side downwards on 3" x 1" glass slides which had been "subbed" in a solution containing 2.5 gr gelatin and 0.25 gr chromealum in 500 ml water at 21° C. The sections to be autoradiographed had been previously mounted on these slides. The films were allowed to dry thoroughly and were then immersed for 4 minutes in a freshly made aqueous solution of the enzyme diastase (2 gr commercial diastase, trade name "Taka-diastase", in 300 ml water). After dissolving, the plates were immersed in distilled water for 5 minutes and were allowed to dry thoroughly. They were then stored in the dark for a time appropriate to the radioactive properties of the object being autoradiographed, after which they were processed in the usual way (1 minute undiluted D-19, 1 minute wash, 10 minutes full-strength F-6, 20 minutes wash; all at 18° C.).

The enzyme solution dissolves some of the gelatin in which the halide grains are suspended and as a result the uppermost grains settle downwards and a film of diminished thickness and greater grain-density is produced. The dissolving technique was used by Comer and Skipper to make a film thin enough to be examined in the electron microscope. The work of these authors was concerned with the detection of -particles emitted by mineral grains embedded in the emulsion and the attainment of adequate resolution was therefore not a serious problem, since the most intimate contact possible between emulsion and specimen was always

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maintained. Further, the origin of the α-particles could be determined to a high order of accuracy by tracing back the characteristically straight and dense tracks. In the present experiments, however, where one is in the main concerned with β-emitters concentrated in tissue sections mounted outside the sensitive layer, reducing the gelatin content should improve the resolution not only by reducing the film thickness, but also, importantly, by reducing the mean grain spacing; for the same number of grains as before will be present in the diminished emulsion volume remaining after dissolution. The increased grain-density may be expected to affect the resolution by:

1. increasing β-track recognisability (a <u>subjective</u> entity) in track autoradiography; or

2. in contrast autoradiography, by increasing the interaction probability in the vicinity of the source.

Among the possible advantages of this technique are the fact that it employs ready-made grains whose adequacy in detecting, as tracks, β-particles with energies up to 150 kev[*] is well known, and renders unnecessary the complex processes of ripening, etc.

Considerable difficulty was experienced in carrying out the experiments and in interpreting the results:

1. In the beginning the slides were coated with

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[*] But see comment on Page 38

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a solution of parlodion in methyl-ethyl-ketone (M.E.K.), since Comer and Skipper had indicated that an impermeable base-layer of some kind was valuable if the film was to be removed later from the mounting plate for electron microscope examination. The parlodion-M.E.K. coating had to be abandoned eventually in favor of the subbing solution mentioned above, as the sticking qualities of the film were very poor unless the composition and thickness of the parlodion-M.E.K. coating were carefully controlled. Even the conventional subbing method was sometimes unreliable, but it is interesting to note that films which had been dissolved survived processing better than the undissolved ones. This was presumably because the gelatin bonded firmly onto the glass slide when reduced to a tacky state by the dissolving agent. The presence of a 5μ gelatin backing on the permeable-base stripping film is disadvantageous in dissolving experiments, since a greater volume of gelatin must be removed in order to obtain the given total film thickness. As we shall see, the prolonged immersion necessary to remove a large volume of gelatin has undesirable effects on the strength and uniformity of the resulting film, and a backing less than 5μ thick would therefore be useful, though it would undoubtedly make routine handling more difficult.

2. The concentration of the enzyme bath is important. If too little diastase is present the quantity of gelatin removed is inappreciable, while if the dissolving

process is too vigorous a film of uneven thickness results,
as shown by the patchy background density in films which
were first processed and then dissolved in a very concentrated
enzyme solution. Experiments showed that the 0.7% (approxi-
mately) by weight concentration finally chosen gave a
thinning effect readily appreciated by the naked eye, without
adversely affecting the uniformity of the film or its mechanical
strength during processing. Unfortunately the optimum con-
centration depends on the type of emulsion employed and on
its age. Thus, British Kodak AR10 stripping-plate emulsion
needed only one-half the dissolving time* of the Eastman
Kodak film, presumably because a different gelatin is used.
Now changes in the gelatin are usually accompanied by changes
in the photographic sensitivity, and since the apparent
resolution is dependent on the sensitivity, at constant
exposure, both the exposure time and the enzyme concentration
must be changed for each emulsion type. There was some evidence
that these adjustments are necessary for different batches
of the same emulsion if they have been manufactured on
different dates or stored for varying lengths of time.

      3. It is very important that all slides remain in
a horizontal position during the dissolving, rinsing and drying
processes. Otherwise the final film is very uneven and may
contain voids where the emulsion has run off the edge of
the plate. In the present work the slides were mounted on a

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*though its backing is 10μ thick

glass rack with shelf-like supports, which was lowered and
raised gently by hand, but in more elaborate experiments
a screw mechanism for lowering the rigidly-held slides into
the reagent baths would be helpful.

4. The test-object used in these experiments was
the plant _Chlorella_ _pyroidosa_ labeled with C-14, supplied
at an activity of 2 μc/mg through the courtesy of Nuclear-
Chicago Corporation. In order to make this rather tenuous
organism visible it was pre-stained with eosin. This led
to a confusing artifact which was not recognised as such in
the beginning and whose cause therefore escaped detection
for some considerable time. It was easily established
afterwards that the eosin staining must be carried out
after all processing is completed.

5. Early experiments concerned with the possible
effects of dissolving on photographic sensitivity were
confused by inattention to the variable swelling of stripping
film when floated on water. The background grain-density
of control films processed after dissolving was compared
with that of similar films dissolved after processing in
the hope of detecting any decreased sensitivity or greater
fogging attributable to the dissolving process. The results
were vitiated by the fact that the degree of swelling and
hence the background density depend on the water temperature,
the floating time, and the drying conditions. In an effort
to standardize, all films were thereafter floated for four

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minutes (if possible -- occasionally a film sinks) before
mounting, but we believe that there is still variation in
the degree of swelling.

6. The results obtained with Eastman Kodak Stripping
Film were difficult to interpret satisfactorily though there
was an apparent improvement in resolution (Fig. 4). The
principal difficulty was the high background which made
suspect any estimate not based on grain-counts. An effort
was made to obviate this difficulty by using the eradication
techniques familiar in nuclear emulsion work. Attempts to
eradicate 5μ emulsions by heating in a moist atmosphere
resulted either in an apparently permanent loss of sensitivity
or in a melted emulsion. Finally Albouy and Faraggi's[11]
scheme of exposure to the vapor of 4% hydrogen peroxide was
resorted to. At first eradication was carried out when the
emulsion had dried at the end of the mounting process, but
it was still difficult to draw any conclusions about the
difference in resolution between dissolved and undissolved
films, for though the background was undoubtedly reduced
it was not entirely removed. In films containing no radio-
active chlorella, however, eradication was complete and it
therefore appeared likely that some at least of the background
was caused by movement during the dissolving process of
grains exposed in the drying period following mounting. In
later work eradication was carried out when the mounting and
dissolving operations were complete. Roughly 24 hours after

removal from the peroxide vapor the films appeared to recover their normal speed and this time was therefore added to the exposure period, which with uneradicated emulsions and the chlorella activity quoted here was about 48 hours.

7. Experiments along these lines were abandoned for two reasons:

a. A film (described previously) of vastly superior resolution had been made.

b. Chlorella is an unsatisfactory test-object for work of this kind. Its main disadvantage is its considerable diameter ($\sim 2\mu$) which is greater than the thickness of the dissolved films. As a result the treated films present a lumpy surface to the viewer, each lump corresponding with a group of organisms over which the film is snugly draped (Fig. 5). The exposed grains immediately above the chlorella are therefore in a plane different from those in the main body of the film. Now the depth of focus of the objective used (95 x, N.A. 1.3) is less ($\sim 1\mu$) than the chlorella diameter and an objective focused at level A in Fig. 3 sees a large number of grains directly over the chlorella and very few in the interstices; an objective focused at level B on the other hand sees

Viewer

Level A
Level B

Glass

Autoradiographic emulsion
Chlorella
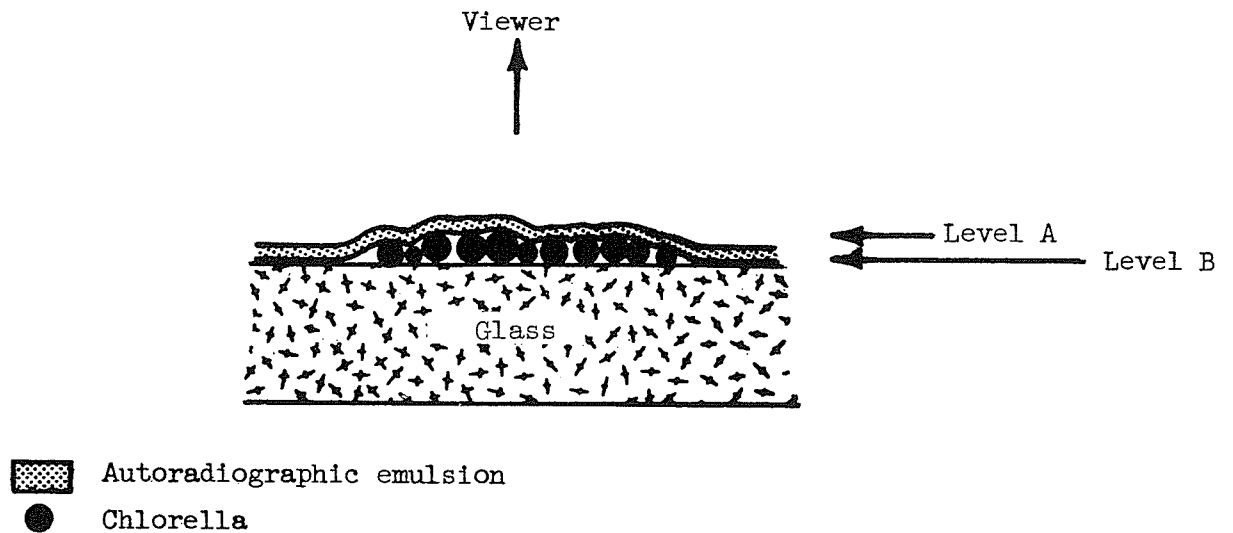
(a)
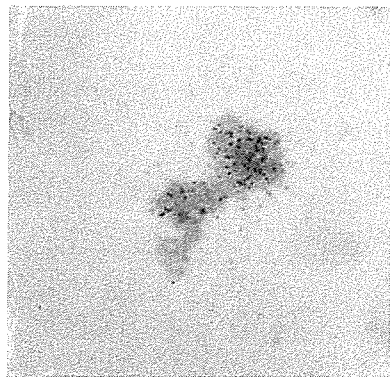
(b)
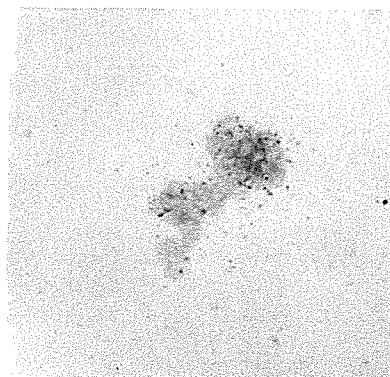
(c)

Fig. 5: Effect of finite object thickness on apparent resolution in
very thin emulsions.

(a) Cross section (not to scale) showing how the emulsion presents
a lumpy surface to an observer viewing it from above.

(b) Typical appearance when the microscope is focussed at level A
in Fig. 3 (a).

(c) Typical appearance when the microscope is focussed at level B
in Fig. 3 (a).

the interspaces dotted with grains. The true resolution could therefore be determined only at large depths of focus, which in practice excludes high-power examination.

C. Continuous Media.

The possibility of individual particle detection using a continuous non-silver medium was investigated concurrently with the experiments reported above.

One of the principal advantages of silver systems is the existence of a multiplicative effect tending to enhance the primary photographic event. No amplification was anticipated in any of the systems described below and the advantages expected to accrue from their use must therefore be great in order to outweigh the serious drawback of non-multiplication. The single advantage of a continuous medium is that it is potentially sensitive along the entire track of a β-particle, rather than at intervals of the order of the grain-spacing. There is therefore a possibility that the entire path, however tortuous, may be rendered visible as a track, a circumstance leading to an extreme resolution which offsets the absence of multiplication. It also lowers the contrast requirements since a continuous track is visible against a denser background than is the case for a track composed of isolated grains. (We may here remark that after considerable work with C-14 β-particles using Eastman Kodak

emulsions, consultation of published information[13] gave rise to serious doubt about the sensitivity of the films to electrons with energies above 80 kev. If, as suspected, the emulsion will not record such particles as tracks, then a fraction of the C-14 β-particles cannot be detected at all* in the vicinity of the emitting atoms, where in the interests of resolution high sensitivity is needed. Correspondence with the manufacturers did not dispel this doubt and British Kodak AR10 emulsion was used subsequently. AR10 emulsion is known to record -particles of all energies as tracks.

The first chemical system tried was the classical ferrous-ferric dosimeter incorporated in a solidified 10% aqueous solution of gelatin. Though the oxidation yield is extremely small the quantity of ferric ion formed during the passage of (say) a single ThB α-particle is large enough to be microscopically visible (a) if it is suitably compounded and (b) if it is localized along the track of the particle. An inert material can be incorporated in the ferrous-gelatin layer to increase the stopping power and thus improve the chances of visualizing the track. The plates were exposed to α- and β-particles and to 200 KV x-rays. They were then differentiated with potassium ferrocyanide which gives a reaction with ferric but not with ferrous ion. Oxidation by atmospheric oxygen prevented satisfactory evaluation of the experiments, and the same difficulty was encountered in

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*Unless the contrast is extremely high

a $FeSO_4$-gelatin system employing 2-4-pentane-dione as a complexing agent for Fe^{3+} ion. No complex is formed when the iron is in the reduced state, while the ferric complex is of high molecular weight and deeply colored. Both systems might have been successful in an inert atmosphere, but this would make routine use difficult, and it was not attempted.

Compounds exhibiting stearic hindrance were then investigated, the supposition being that an atom which is prohibited by its size from remaining at the normal separation from its neighbors in the molecule is correspondingly weakly bound and therefore more vulnerable to dislodgment by radiation. The compounds tried were bromoform and iodoform and it was proposed to use the leucobase of paraphenylenediamine (P.P.D.) to detect the halogen set free from them by radiation. The bromoform system was made by dissolving P.P.D. in bromoform and incorporating the solution in aqueous gelatin with addition of amyl alcohol for stabilization of the emulsion. The aqueous gelatin was omitted in the iodoform system and replaced by amyl acetate. No sensitivity to particulate or x-radiation was observed.

A system using stoichimetric quantities of sodium iodide and iodate with P.P.D. was also unsuccessful as a track detector in the solid form. Similar negative results were obtained with solutions of photographic "hypo" in gelatin. Deposition of sulphur linked to the molecule by a vulnerable double bond was expected but not observed. The

mercuric-chloride ammonium oxalate system also failed as did
attempts to produce the chloroform-bromcresol green system in
solid form.

Lastly the light-sensitive diazo-system of Alink,
Kennig and Dippel[14] was tried in some detail using the
double salt p-diethylaminobenzene - diazonium chloride -
$ZnCl_2$ supplied by Antara Chemicals of New York. No radiation
response was elicited and Dr. Dippel later confirmed that he
had had a similar experience with ionizing radiations.

D. Gelatin Bichromate Films.

These films were manufactured by using 5% gelatin
in water with a small amount of ammonium bichromate. Slides
were dipped in this solution and allowed to dry slowly.

These films were exposed to a slit of light and
then developed by rinsing in warm water. The gelatin washed
off the slide, except for the area which had been exposed
to light. The results were nice and clean and there was
absolutely no background. However, the system, as may be
expected, is not very sensitive. It took 5-10 minutes
exposure to light to give really sharp results. Exposures
of only 1-2 minutes could be seen on the gelatin, but would
disappear with development -- no matter how little development
was used. Also, when exposed to x-ray, this film showed only
a suggestion of an autoradiograph -- a really sharp grain was
never obtained. The system was not pursued further.

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APPENDIX I

Publications By Participants In Study Of

High Resolution Detection Of Radiation

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6. High Resolution Autoradiography in Metallurgy. H. J. Gomberg et al. Project 2029, Interim Report, Engineering Research Institute. February, 1955.

7. Theoretical Aspects of Autoradiography. Proceedings - Conference on Autoradiography. Oak Ridge Institute for Nuclear Studies. June, 1953.

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