TABLE I. Effect of Niacin Deficiency on Rate of Alcohol Metabolism.

Group	No. of rats	Deficient or pair fed	Wt in g (range, avg)	Age in days (range, avg)	Fed or fasted	Rate of metabolism (mg/kg/hr), avg ± S.D.	p values
I	6	Def.	40-65 49.2	44-54 49.3	Fed	493 ± 39	<.10 >.05
	6	P.F.	46-68 54.0	44-54 49.3	"	401 ±106	
II	6	Def.	38-70 50.3	45-55 49.7	"	461 ± 25	<.01
	6	P.F.	46-66 52.2	45-55 49.7	Fasted	372 ± 63	
III	7	Def.	46-53 48.1	53-64 59.7	"	370 ± 41	<.01
	7	P.F.	46-53 50.9	53-64 59.7	"	313 ± 21	
I, II, III	19	Def.	38-70 49.1	44-64 53.3	—	438 ± 64	<.01
	19	P.F.	46-68 52.3	44-64 53.3	—	359 ± 76	
I, III	13	Def.	40-65 48.6	44-64 54.9	—	427 ± 74	<.05 >.01
	13	P.F.	46-68 52.3	44-64 54.9	—	353 ± 84	

rate of alcohol metabolism in spite of the fact that DPN is generally conceded to be involved in conversion of alcohol to acetaldehyde.

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Preparation of Coated Radioautographs by Dipping Sections in Fluid Emulsion.* (23380)

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In 1946, the coating method of radioautography was developed in collaboration with Bélanger(1). The intimate contact provided by this method between the photographic emulsion and the radioactive tissue section insured a high resolution, which has not been improved by any technical procedures, except by the use of high contrast "nuclear" emulsions. In 1947 it was proposed to mount the section directly on a photographic plate(2) or to place a stripping film over the section(3). A high resolution could also be obtained with these procedures(4), but the coating method(1,5) has been preferred by the present authors because of the speed with which large batches of slides may be handled. More recently, Joftes and Warren proposed a method in which the slide is dipped in melted emulsion(6). While several steps of the technic were felt to be cumbersome or unnecessary, the dipping itself is simple and requires a minimum of equipment. An attempt was, therefore, made to introduce a "dipping" step into the coating method routinely used here. The results proved to be highly satisfactory and the technic will now be described in detail.

Slides bearing histological sections, either stained or unstained, are placed in a 1% solution of celloidin for a few seconds ("Tissue Imbedding Solution" No. M 4700, manufactured by Randolph Products Co., N. J., di-

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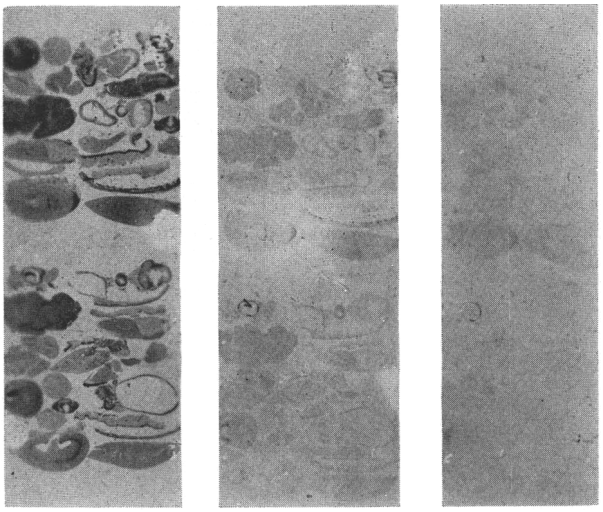
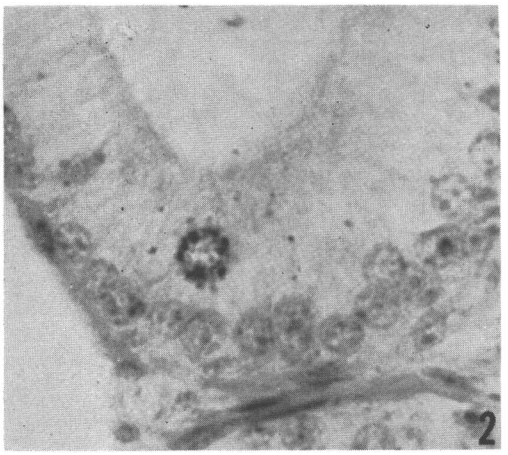


FIG. 1. Unstained radioautographs of tissues from mouse sacrificed 1 hr after inj. of S^{35}-methionine. Exposure at various humidity levels. After exposure in a dry atmosphere (left), reactions were intense, while at 40% humidity (center) reactions were weak and at 95% humidity (right) reactions were lacking. (Slightly darkened areas visible on right hand side are not due to reactions but to traces of picric acid retained in the Bouin fixed tissues.)

luted 30 times with a 1:1 mixture of ether and absolute alcohol). After drying for 10-15 minutes, the slides are placed in celloidin again for a few seconds. The celloidin coats preserve the staining to some extent during development and fixation, and also prevent the direct contact of the photographic emulsion with the tissue, thus minimizing chemical fogging by non-radioactive tissue components or dyes(4). After drying overnight at room temperature, the celloidin coated slide is smeared with a small drop of a 1:1 egg albumin:glycerine solution on either side of the sections, a procedure by which the emulsion is kept in place during development and subsequent handling.

The steps to follow are carried out at about 3 feet from a Wratten safelight No. 2 in a completely light-proof dark room. The temperature of the room is maintained at 60°F (16°C) and the humidity kept between 10-20% by leaving an open dish containing Drierite (a drying agent) in the room. The flask containing the stock solution of gelled emulsion (so far, bulk Eastman Kodak NTB 2 and NTB 3 have been used) is removed from a refrigerator kept in the dark room. A volume of emulsion estimated at about 60 ml is scooped out with a porcelain spoon and dropped in a Borrel tube standing in a water bath at 40-45°C. The emulsion is left in the water bath for at least 1 hour, by which time it is fluid and reasonably free of air bubbles (which otherwise produce artefacts in the emulsion coat). It was found useful, although not essential, to warm the slides on a hot plate at 40°C to allow a more uniform spreading of the emulsion. Then, the slides held at the label end are dipped for 1-2 seconds in the melted emulsion.

Attempts to use emulsion at 33°C failed as the emulsion coat was thick and unevenly distributed, while at 42°C and 60°C the layer had the desired thinness and uniformity. However, since temperatures above 50°C were found to induce bubble formation and increase the danger of fog, a temperature of 40-45°C was finally selected. After withdrawal of the slide from the fluid emulsion, the excess is allowed to drain into the container and the back of the slide is wiped clean with soft paper. The slide is then allowed to dry in a vertical position. It has been found that the emulsion layer hardened more uniformly when the slides were kept vertical than when laid flat. Drying is complete when the slide has been left standing for about 1½ hours (with the safelight turned off).



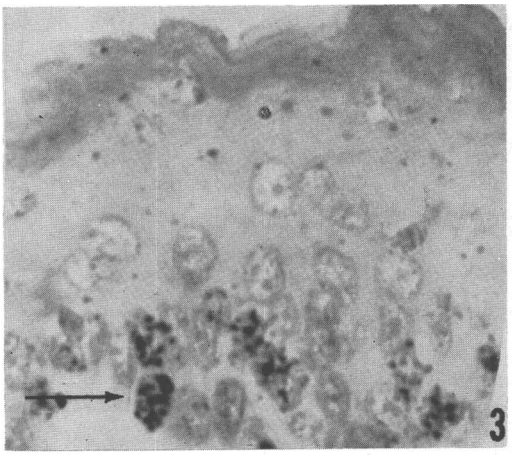


FIG. 2. Radioautograph of epididymis from mouse sacrificed 8 hr after inj. of H^3-thymidine. Hematoxylin and eosin stain. The nucleus of an epithelial cell in prophase shows a positive reaction. (In this, as in many epithelia, the nuclei move towards the lumen soon after prophase starts.) × 800.

FIG. 3. Radioautograph of tongue epithelium from mouse sacrificed 24 hr after inj. of H^3-thymidine. Hematoxylin and eosin stain. A positive reaction is visible over several of the nuclei in the basal layer. × 800.

The thickness of the emulsion was estimated to be 4 μ under the above conditions, but could be increased by dipping the slides 2, 3 or more times.

In seeking optimum condition for exposure, the influence of humidity on the radioautographic image was investigated as follows. A number of unstained sections from mice sacrificed 1 hour after injection of S^{35}-methionine were coated by dipping in NTB 2 emulsion as described above. The slides were distributed in 3 light-proof boxes kept at 5°C which, in addition, contained respectively: 1) Drierite to reduce the humidity close to 0%; 2) a beaker filled with a saturated solution of $CaCl_2 \cdot 6H_2O$ to yield an atmosphere with 40% humidity; and 3) a beaker filled with a saturated solution of $ZnSO_4 \cdot H_2O$ to obtain 95% humidity. After 7 weeks, the slides were developed and fixed simultaneously. It may be seen (Fig. 1) that the radioautographic reaction was intense in a dry atmosphere, weak at 40% humidity and absent at 95% humidity. Similar results were obtained with thyroid sections containing I^{131} and exposed for only 5 days, so that the inhibitory effect of humidity is just as pronounced after a short exposure. Since in both experiments the response was maximal in the absence of humidity, it was decided to expose all further radioautographs in a dry atmosphere. Routinely, the slides are stored in black plastic boxes, containing a perforated stainless steel capsule filled with Drierite. The box is sealed with black adhesive tape and kept standing on edge in the refrigerator (5°C) so that the slides are exposed in the horizontal position, emulsion side down.

After exposure, the preparations are developed for 1½ minutes in Eastman Kodak Dektol developer (D-72 type). Fixation is carried out for 10 minutes in acid fixer with hardener. The slides are then washed in running water at 18°C for 15 minutes and dehydrated in 95% alcohol and 2 changes of absolute alcohol.

For mounting radioautographs in permanent form, the slides are taken through absolute alcohol, rinsed in acetone for 5 minutes, placed for 5 minutes in a 1:4 acetone dilution of "vinylite cement" (General Cement Mfg. Co., Rockford, Ill.), and finally allowed to dry overnight at 60°C in the vertical position. The next day, the radioautographs are mounted by adding a drop of Canada balsam and covering the preparation with a coverslip (Fig. 2 and 3).

Summary. A simplified coating method for radioautography has been described in which the radioactive slides are dipped in melted

photographic emulsion. After exposure, the developed emulsion is protected with a coat of vinylite and mounted under coverslip.

Note added in proof: a dipping method of radioautography was also found in the German literature: Kolousek *et al., Acta Histochemica,* 1956, v3, 328.

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Utilization of Methylglyoxal in Animal Tissues. (23381)

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In spite of the difficulty of detecting methylglyoxal (MG) in the presence of the highly active enzyme glyoxalase, Neuberg and Kobel(1) isolated the compound from products of fermentation of hexose diphosphate (HDP) by dried yeast. MG is also formed from HDP by mixed preparations of muscle and pancreas (Tonniessen and Fisher(2), Ariyama(3)), and by acetone powders of rat liver (Artom(4)). Geiger and Rosenberg(5) detected MG in urine of polyneuritic dogs and in urine and cerebrospinal fluid of infants suffering from acute toxic dyspepsia; and Salem(6) could demonstrate MG in urine of thiamine-deficient rats. It appears therefore that MG is produced from HDP and can be detected under conditions, in which activity of the glyoxalase system is reduced or suppressed.

The aim of the present paper is to examine the possibility that MG could be converted enzymatically to HDP, *i.e.* to investigate whether the reaction: HDP→MG + Inorganic phosphate (Pi) can be considered as a reversible reaction.

Methods. MG was prepared from acetone by slight modification of the method of Riley, Morley and Friend(7). HDP was supplied by L. Light, Ltd., as the Ca salt. Fresh rat liver was weighed, ground with sand in a mortar, then extracted with one part of water and with occasional stirring for 30 minutes in the refrigerator. The mixture was centrifuged, and the supernatant used as a source of the enzyme. Pi was determined according to Fiske and Subbarow(8) and MG according to Salem(6). Two methods (a) and (b) were used to identify the compound resulting from incubation of liver preparation with MG: (a) Ba soluble and Ba insoluble phosphorylated compounds were fractionated, as described by Umbreit *et al.*(9), and hydrolysis curves were determined according to Lohmann(10); (b) descending one-dimensional paper chromatograms were made with trichloroacetic acid:acetone (35/65 v/v) as solvent. Molybdic acid method for phosphate esters (Hanes and Isherwood(11), Axelrod (12)) was used to detect HDP. Apparatus and working details were as described by Dent(13).

Results. Changes of Pi and MG during incubation: 1 ml of liver extract was incubated with 0.5 ml of 1:5 kidney extract for 2 hours at room temperature, to inactivate the glyoxalase system. Then 2 ml of 1% neutralized MG solution, 2 ml of borate-boric acid buffer, pH 7.0, and 0.2 ml toluene, as an antiseptic, were added. A similar sample was prepared, except that MG was omitted. Both samples were incubated at 37°C for 24 hours. The protein was precipitated with 10% trichloroacetic acid, and the amounts of Pi were determined on supernatants before and after incubation (with or without MG). From Table I, it is apparent that when MG was incubated