was associated with bacteremia.‖ When avirulent live bacteria were injected, the recovered organisms were either of the strain inoculated, or were apparently autogenous bacteria. However, when heat-killed bacteria were introduced, the recovered organisms were of autogenous origin only. It is known that bacteremia, identified with normal bowel inhabitants, is often an integral part of the syndrome observed in animals dying from whole body irradiation. In this instance, however, bacteremia and death were induced in animals exposed to sublethal doses of irradiation and subsequently inoculated with live avirulent, or even dead organisms generically different from the bacteria normally present in the intestines of these animals. This observation may be of importance under conditions where irradiation is a factor and vaccination is indicated.

‖ Complete identification of isolated lactose fermenting cultures was not made.

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Autoradiograms of Irregular Surfaces.* (18685)

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In studies of shell formation using Ca^{45} and P^{32} it became necessary to follow isotope distribution on the curved inner surfaces of mollusc shells. For accurate localization of radioactivity on such surfaces by means of photographic emulsions close contact between the emulsion and the surface is necessary. Photographic plates and cut film which are satisfactory for flat surfaces will not give sufficiently close contact with curved and irregular surfaces. Liquid emulsion, which might be used, has not proved suitable in our experience in providing a layer of uniform thickness; and stripping film is not sufficiently flexible to conform to irregular surfaces. We have found that emulsion sheets removed from x-ray film will serve very satisfactorily. The emulsion layer is uniform in thickness and will faithfully follow and adhere to all contours. This material is also useful for making autoradiograms of bones as well as shells. Details of the method are illustrated below with bones of the guinea pig.

Eastman Industrial X-ray Film, Type A, in strips 1⅞ x 16 inches was used. This film is more sensitive than Type M and less sensitive than No-Screen and Type K. Because of its smaller grain size the resolution is better than the latter two(1,2). All operations are carried out in a darkroom using a Wratten safelight, Series OA. After cutting the film into pieces of appropriate size they are placed in distilled water at 30°C for about 5 minutes to soften the emulsion. By means of a piece of plastic or glass with a polished edge the emulsion can now be completely removed in a sheet by beginning at one end of the film strip and pushing the emulsion ahead of the instrument. With a satisfactory edge the sheet of emulsion is removed without defect and with none remaining on the backing. The emulsion is then placed in distilled water at 20°C where it will toughen and wrinkles will disappear. The emulsion is applied by floating it onto the surface to be studied. As the object is lifted from the water the sheet of emulsion will become closely applied to all contours (Fig. 1 and 2). Any wrinkles can be smoothed with a camel's hair brush. Leaching of radioactive material from shell and bone during the exposure to distilled water and to the wet emulsion will presumably be minimal.

After the emulsion is dry the preparations are stored in light-tight boxes for a suitable

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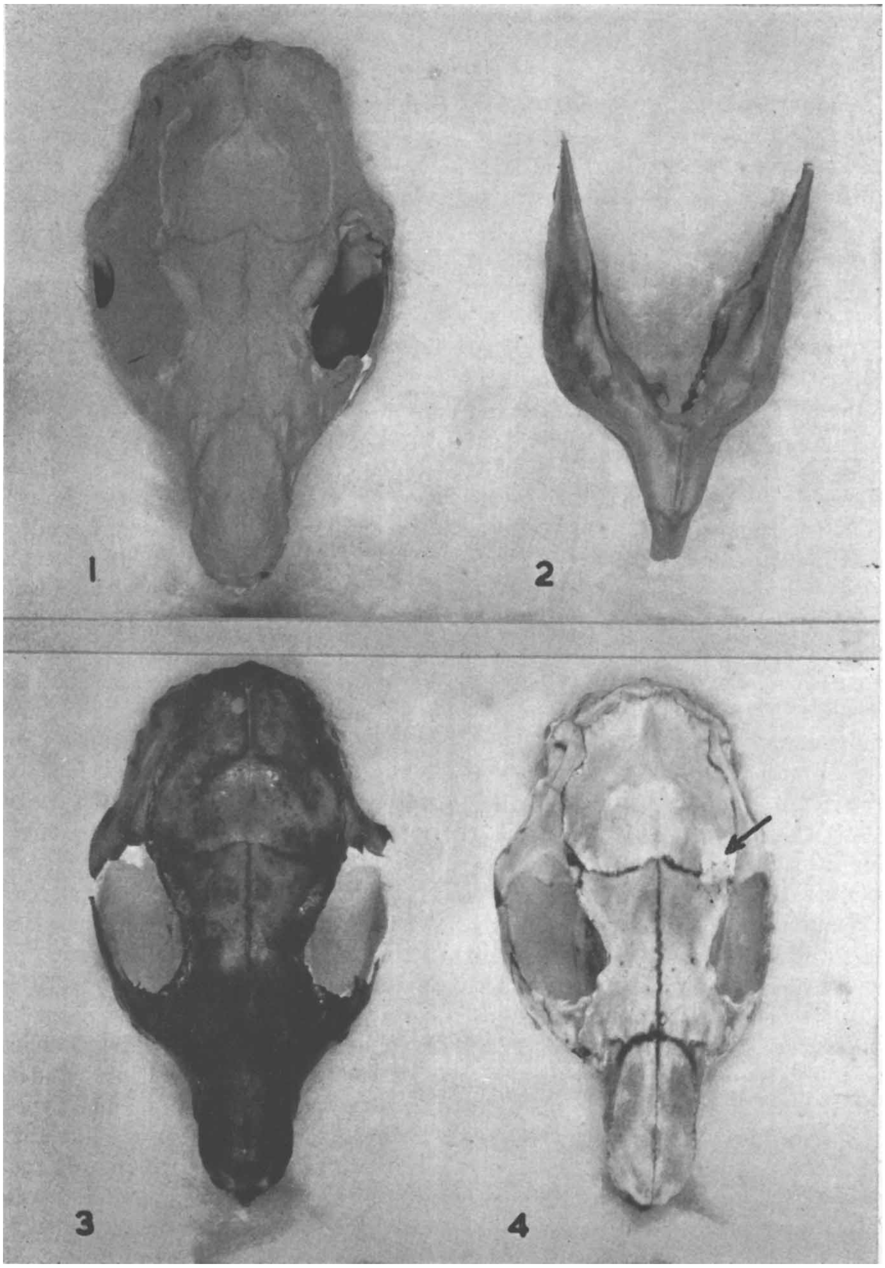

FIG. 1 and 2. Guinea pig skull and lower jaw covered with sheet emulsion from Eastman Industrial X-ray Film, Type A. Emulsion left uncleared to show its conformity to contours.

FIG. 3. Autoradiogram of guinea pig skull showing non-uniform distribution of P^{32}. A similar distribution of P^{32} has been found in 8 skulls. Eastman Industrial X-ray Film, Type A. Highlights interfere somewhat with the accurate representation of darkening in this photograph. 100 μc P^{32} injected intraperitoneally; animals sacrificed after 20 hr. Skulls heated very briefly in boiling water for cleaning. Emulsion exposed 24 hr.

FIG. 4. Non-radioactive guinea pig skull covered with emulsion, developed and cleared. Black lines at sutures are the result of chemical or physical action. Arrow indicates an area including a suture from which the emulsion has been removed.

exposure time. The emulsion is then developed in D-19 developer(2) while still on the specimen. Fixation is carried out in the following alkaline solution: sodium thiosulphite, 960 g; sodium sulphite, desiccated, 50 g; water, 4 l. Since the solutions are alkaline except for a very brief acid stop-bath, no decomposition of shell or bone will occur. Development of the autogram while on the specimen obviously facilitates the accurate localization of radioactive areas (Fig. 3). However, qualitative visualization of regional differences in radioactivity of a specimen is, of course, limited to uniformly light colored material. Quantitative data may be obtained by counting developed silver grains while the emulsion is still on the specimen or by removing it to a glass plate and making densitometer measurements. For the latter procedure reference marks may be made on the emulsion and the underlying material with a pointed instrument and the emulsion sheet removed immediately after development while still moist. This is easily accomplished by loosening at the edges. Radioactive ink (3) applied to the shell or bone will also provide reference points on the isolated emulsion.

Counting of silver grains in the emulsion on radioactive oyster shells may be facilitated by making photomicrographs of the intact preparation using a 20 x objective and a 15 x ocular and a Whipple disc(4). The negatives are then projected with a photographic enlarger. Quantitative measurements of Ca^{45} and P^{32} in oyster shells have been made in this way. For surfaces with more extreme contours the emulsion may be removed for photographing.

During removal from the backing the emulsion increases in linear dimensions by about 25%, a property shown also by stripping plate emulsion(1). We have studied this by ruling small squares on the emulsion prior to removal and making measurements after consecutive steps in the procedure. Such measurements show that the stretching which occurs after removal of the emulsion from the backing is uniform in all directions. No apparent stretching or distortion occurs as a result of applying the film to a curved surface, subjecting it to development procedures, or by removing the developed emulsion to a glass plate. However, localized stretching of the emulsion probably occurs in conforming to marked curvatures.

Because of direct contact between the emulsion and the specimen the possibility of darkening through physical or chemical action must be considered and suitable control exposures made on non-radioactive material. Non-radioactive oyster shells coated with emulsion and run through the entire procedure exhibited no darkening of the emulsion. However, in a series of 14 non-radioactive guinea pig skulls 11 showed varying degrees of darkening at sutures. The most extreme case is illustrated in Fig. 4. Darkening also occurred over certain areas of the lower jaw. Further investigation of the cause of this darkening would be interesting but is beyond the scope of this study. The elimination of this effect presumably could be approached by the application of film lacquer or some other inert substance to the surface to be autographed. However, this would reduce soft beta radiation from the surface; and if the thickness of the applied material were not uniform, the autogram would not give a true record of the radioactivity.

Summary. 1. Autoradiograms of irregular surfaces of mollusc shells and curved surfaces of bone can be made without difficulty by applying sheets of emulsion removed from Eastman Industrial X-ray Film, Type A. The emulsion is developed while on the object, facilitating the localization of radioactive areas, and may be removed for densitometric measurements if desired. 2. Darkening of the emulsion may occur as a result of direct contact with sutures and certain definite regions of non-radioactive bone. Accordingly, in making autoradiograms by this method control exposures on non-radioactive bone are required to evaluate this effect.

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