more than one isolation of virus from the affected mice, but the great similarity of the disease in the spontaneously-affected and the experimentally-infected mice would seem to be a good indication that the virus isolated was actually responsible for the epizootic.

Summary. A spontaneous epizootic of encephalomyelitis which killed 62% of 240 uninoculated mice is described. A virus was isolated which resembles GD VII virus in its properties, and which is antigenically identical or very similar to the GD VII virus.

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Detection of Phosphatase Activity in Polarized Light Following Glycerophosphate Incubation.* (18745)

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The differences in the exact localization of phosphatase activity such as reported in the literature are due to several factors. Among these is the fact that in the Gomori technic of histochemical detection(1), the white precipitate formed is transformed into a black mass by the cobalt nitrate-ammonium sulfide treatment. Unfortunately such a treatment produces also some artificial blackening part of which has already been recognized, such as that of the ground substance of the cartilage. On the other hand, in the black areas of phosphatase activity, it is possible that some local changes in the amount and position of the precipitate might take place during the transformation of lead or calcium phosphate into sulfide.

In order to control these two factors of error, one might try and examine the initial precipitate, a thing which is made easy by using polarized light and an environment which will not reduce the anisotropism of the mineral salts.

Material and methods. Rat and hamster tissues were fixed in 80% alcohol or cold acetone. The tissues were embedded routinely in paraffin or low viscosity celloidin, according to a technic described previously(2). Sections of already mineralized material such as bones and teeth were treated with a buffer decalcifying solution of formic acid and sodium citrate at pH 4.9 according to Greep, Fischer, and Morse(3). By treating 10 mμ sections instead of blocks, the time in that solution was reduced from a matter of days to a maximum of 15 minutes. Incubation was carried out at 37°C in Gomori's solutions of sodium alpha-glycerophosphate at pH 5.0 and pH 9.4 for acid and alkaline phosphatase respectively, and also other substrates such as glucose-1-phosphate, adenylic acid, ribonucleic acid, desoxyribonucleic acid and sodium desoxyribonucleate.

Following 3 hours of incubation, the sections were brought to room temperature and dehydrated in graded alcohols and finally mounted with a coverslip in 5% celloidin in alcohol-ether. The controls were similarly treated and all preparations had to be thoroughly dry before examination. In examining these preparations regular polarizing equipment has been used but in comparison, it has been possible to do just as well with a simple addition to a monocular microscope of 2 pieces of Polaroid material of type JG30FAP commonly used in antiglare goggles. These pieces are cut to appropriate sizes to fit the filter slot at the base of the condenser (polarizer) and the inside of the eyepiece (analyser) where the last one rests on the metal insert midway between the top and the bottom lenses. With this equipment, a red extinction of the field is obtained when the

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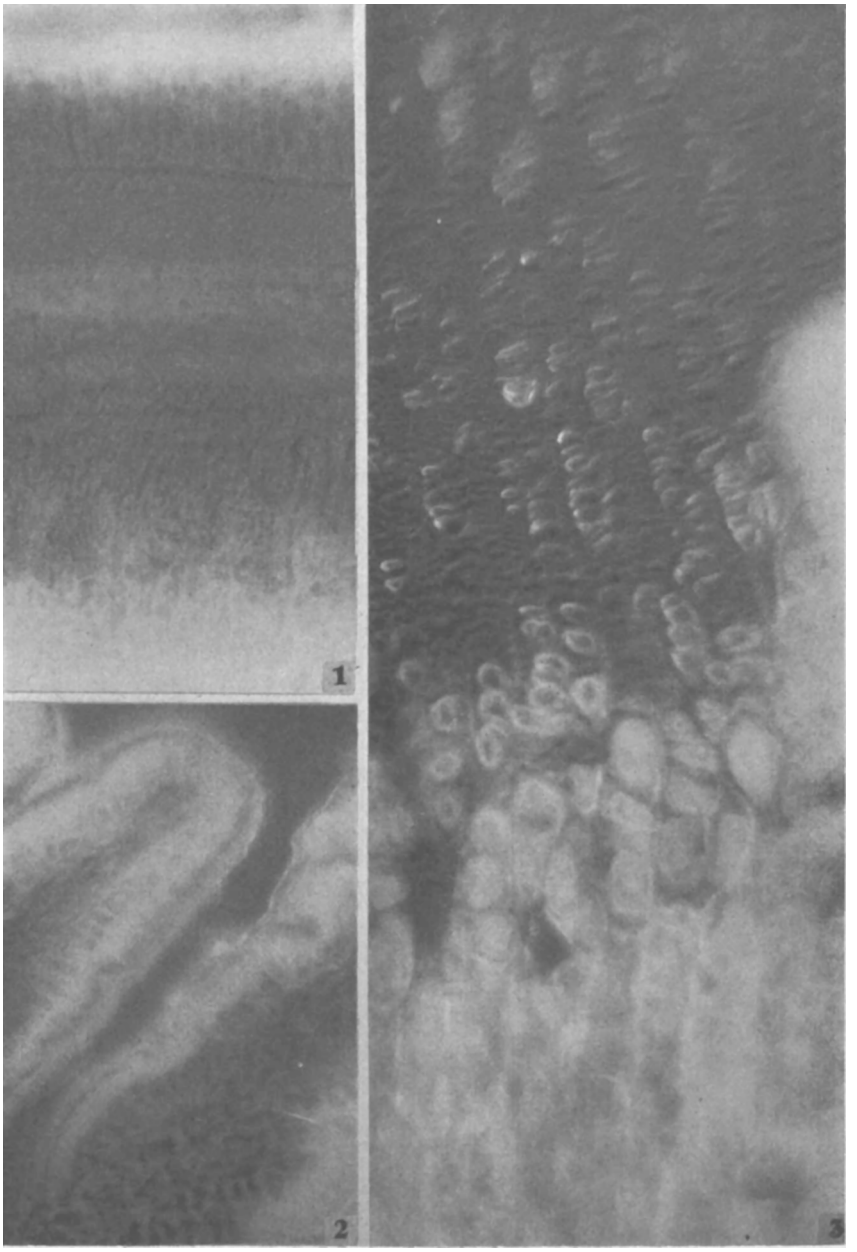

FIG. 1. First molar, upper jaw of an 8-day-old rat. Alkaline phosphatase after demineralization; glycerophosphate, 3 hr. ×315. From the top down can be seen the stratum intermedium of the enamel organ (white), the ameloblasts (dark), the enamel and dentine, the odontoblasts (dark) and the proximal region of the pulp (white).

FIG. 2. Villi of the ileum of an 8-day-old rat. Alkaline phosphatase, 3 hr. ×368. The epithelial cells show an extranuclear reaction (white) progressively increasing from the base towards the tip.

FIG. 3. Epiphyseal plate of the humerus of a 6-day-old hamster. Alkaline phosphatase after demineralization. 3 hr, glycerophosphate. ×315. The hypertrophic cells of the cartilage show an increasingly intense reaction. The bone spicules (lower part of the picture) are intensely positive as well as "Ranvier's notch" (in the middle of the picture, extreme right).

polarizing planes are crossed and the precipitates show in the complimentary yellow-green color.

By a procedure similar to that of Dempsey, Wislocki, and Singer(4) quantitative estimates of the amount of precipitate have also been obtained by comparison of the light absorption power of the precipitate of different areas in white light as the section is projected on the ground glass of a photographic apparatus and the light absorption is measured under comparable conditions with a sensitive densitometer. In undecalcified material, the natural mineral deposits also show with great detail, under polarized light in conditions such as mentioned above.

Observations. Part of the results will be reported elsewhere along with other studies of the phenomenon of mineralization of bones and teeth. For the present purpose, the attached pictures demonstrate the following facts:

Against the dark background, the areas of phosphate precipitates are seen in great detail. In the developing tooth (Fig. 1) the alkaline phosphatase reaction is visible behind the ameloblasts and also behind the odontoblasts. Thin lines of precipitate are visible between the distal ends of these 2 groups of cells. The substance of the dentine and of the enamel are both negative.

In the villi of the intestinal epithelium (Fig. 2) the reaction is present at the apical portion of the cell and it increases in intensity from the base to the tip of the villi. The mucosal glands are negative. In the areas of lesser activity in the villar epithelium, it is possible to see that the reaction is truly a cytoplasmic phenomenon.

At the epiphyseal plate of the long bones (Fig. 3), the phosphatase activity is present in the enlarged cells of the cartilage, over the new bone spicules and on the right hand side of the picture, over this terminal zone of the periosteum known as "Ranvier's notch." In the cells of the cartilage where the reaction is minimal, it appears to be definitely limited to the cytoplasma.

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Comments. As compared to the localizations reported in the literature, several of the preceding descriptions are at variance. When the standard cobalt nitrate-ammonium sulfide treatment was applied to preparations similar to those described above, black precipitates were visible in other areas, notably over the nuclei of cells close to sites of phosphatase indicating possible displacement and adsorption of the precipitate as a result of this operation. Other areas were black in control preparations incubated without glycerophosphate. Such were to variable extents, the organic matrix of the dentine, enamel, bone and cartilage and the keratinized portion of the skin. All these areas were obviously negative in polarized light. On the other hand, certain tissues are known to have anisotropic properties on account of their chemical composition or simply on the basis of their physical arrangement. These were negligible in the preparations studied so far but the possibility of artefact must always be searched for in control slides.

In areas of actual precipitate, details are greatest in border regions where the phosphatase activity is minimal. They become blurred in areas of maximal deposition of calcium or lead salt (Fig. 3). Preliminary studies with the present technic demonstrate the following facts: (1) phosphatase activity in growing bones and teeth is localized to areas which are not necessarily those of mineral deposition (stratum intermedium of enamel organ, pulp of the teeth, Ranvier's notch in long bones); (2) phosphatase activity originates in the cytoplasma of cells; (3) it increases with the differentiation of the cell (enlarged cartilage cells and epithelium of the villi).

Summary. A method of detection of phosphatase activity is described whereby some artefacts of diffusion, adsorption and nonspecific sulfide blackening are dealt with by examination of the original calcium or lead phosphate precipitates in polarized light. This technic demonstrates also the presence of natural mineral deposits in undecalcified material. Comparable estimates of the amount of salt deposited is made with the help of a densitometer.

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Toxicology of Podophyllin.* (18746)

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When the colchicine-like effects of podophyllin were demonstrated(1,2), it was anticipated that the resin of podophyllin would be investigated in the treatment of cancer of the skin. Subsequent exhibitions of selective damaging effects of podophyllin and podophyllotoxin on mouse tumor cells *in vitro*, confirmed by *in vivo* studies in tumor bearing mice(3-5) stimulated the initiation of several clinical trials now in progress(6-8). Phillips, Chenowith, and Hunt(9) found the LD_{50} of podophyllotoxin to be 33 mg/kg for mice and 15 mg/kg for rats. Greenspan and Leiter(10) reported the maximum tolerated single dose of podophyllotoxin in mice to be approximately 30 μg/g.

The purposes of this investigation were: 1. To compare the toxicity of podophyllin with that of colchicine in mice. 2. To obtain additional toxicity data in rats on podophyllin and its components as a prelude to the clinical use of podophyllin in the treatment of cutaneous carcinoma(6). Podophyllin U.S.P. is a complex resinous mixture which contains: podophyllotoxin, the component principally responsible for the drug's curative effect on condyloma acuminatum(2,11,12); quercetin; and 2 recently isolated crystalline components, designated as alpha-peltatin(13) and beta-peltatin(14). Inasmuch as alpha-peltatin and beta-peltatin were unobtainable, our study was limited to podophyllin; podophyllotoxin and its 2 products of alkaline hydrolysis, namely, (1) picropodophyllin and (2) podophyllic acid; quercetin; and depodophyllotoxinized resin. Detailed descriptions of the aforementioned 6 materials are recorded elsewhere(12,15).

Method. Material for parenteral administration was prepared by dissolving each test substance, with the exception of picropodophyllin, in absolute alcohol. One cc of a one or 10% alcoholic solution of the test substance was added to 9 cc of a 10% gum acacia suspension or to a 0.5% methocel suspension. Picropodophyllin was suspended in water with methocel. Test materials for oral administra-

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