linoleic acid, but olive oil, which has about 75% oleic acid, fails to produce encephalomalacia, whereas lard, which contains about 40% oleic acid, balanced by an approximately equal amount of saturated fatty acids, does provoke cerebellar pathology. Similarly, ability of coconut oil to enhance appearance of encephalomalacia seems to be related to its high content of lauric and myristic acids, whereas butter, having mostly a mixture of oleic, palmitic, myristic, and stearic acids, has no such effect.

Summary. Corn oil or lard from which tocopherol had been removed, promoted chick encephalomalacia, whereas coconut oil, butter, linseed oil, and cod liver oil did not produce symptoms, and olive oil had a questionable effect. Dietary combinations of 2% corn oil with either 8% coconut oil, lauric acid, myristic acid, or a mixture of saturated fatty acids like coconut oil significantly increased the incidence of encephalomalacia over 2% corn oil alone, while 6% linseed oil, cod liver oil, or oleic acid inhibited the effect of 4% corn oil. Olive oil, butter, fatty acids like butter, palmitic acid, and stearic acid had no net effects upon the incidence of encephalomalacia induced by corn oil. The intake of linoleic acid appears to be a primary factor in the etiology of encephalomalacia, but some of the other fatty acids may secondarily increase or decrease this effect.

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A Method for Detection and Estimation of Penicillin in Poliomyelitis Vaccine.* (25255)

R. Q. THOMPSON, C. THOMPSON AND C. T. PUGH (Introduced by O. K. Behrens)
The Lilly Research Labs., Eli Lilly and Co., Indianapolis, Ind.

During the manufacture of poliomyelitis vaccine, penicillin is introduced into the tissue culture medium at a concentration of 200 units/ml. Most of this penicillin is either removed or degraded during the processing of the vaccine. In recent medical literature there has been occasional reference to development of symptoms suggestive of penicillin sensitivity as a result of injecting poliomyelitis vaccine. However, data obtained during field trials in which this vaccine was tested, as well as experiences with subsequent mass immunizations, have shown that the incidence of sensitivity reactions is extremely low. Only 0.4% of subjects vaccinated in field trial studies, showed reactions to the vaccine. If only major reactions are tabulated, the incidence decreases to 0.004% (1). Although most of the penicillin introduced into poliomyelitis vaccine is removed or degraded to form microbiologically inactive products, it is of interest to determine residual pencillin in the finished vaccine. Microbiological assay indicated that residual penicillin would be present at low concentration. Because of this low concentration and because of presence in the vaccine of preservatives that interfere with microbiological assay, special precautions

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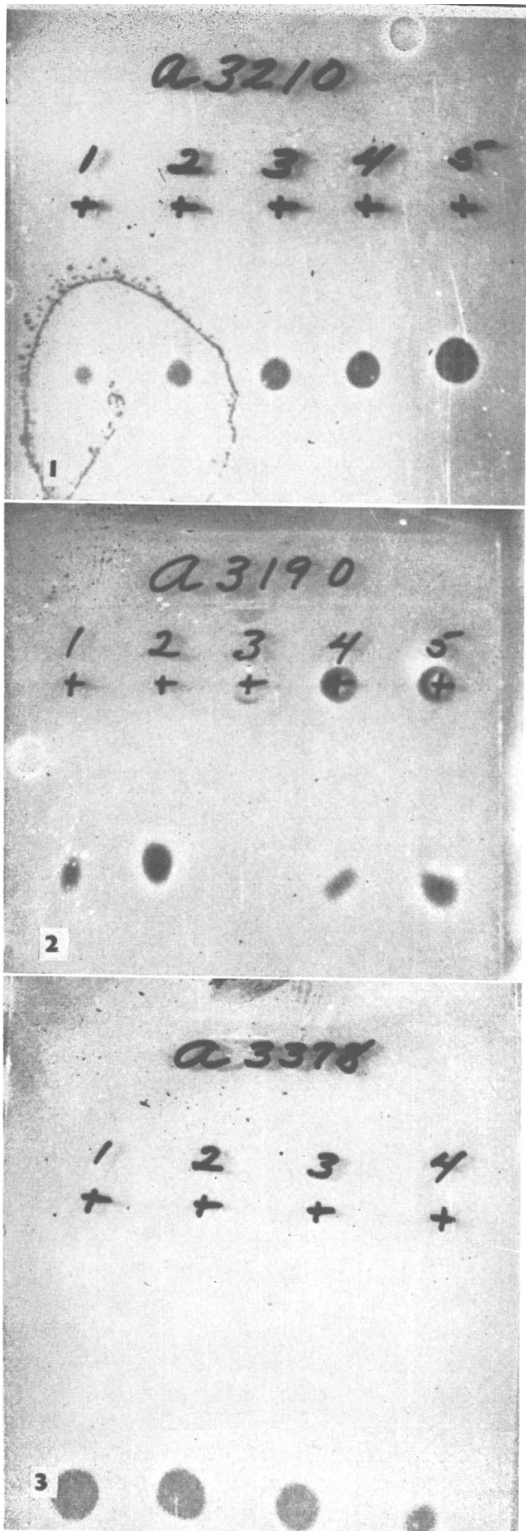

FIG. 1. Bioautograph of penicillin G. Lane 1, 0.0025 unit; Lane 2, 0.005 unit; Lane 3, 0.0075 unit; Lane 4, 0.01 unit; Lane 5, 0.02 unit. The plus signs indicate point of sample application.

FIG. 2. Bioautograph of penicillin recovery from poliomyelitis vaccine. Lane 1, 0.005 unit penicillin G; Lane 2, 0.01 unit penicillin G; Lane 3, vaccine extract (100 μl) eq. to 5 ml vaccine; Lane 4, vaccine extract (100 μl) eq. to 5 ml vaccine to which 0.001 unit penicillin/ml had been added; Lane 5, vaccine extract (150 μl) eq. to 7.5 ml vaccine to which 0.001 unit penicillin/ml had been added.

FIG. 3. Bioautograph of penicillin recovery from poliomyelitis vaccine. Lane 1, vaccine extract (10 μl) eq. to 0.5 ml vaccine to which 0.05 unit penicillin/ml had been added; Lane 2, 0.025 unit penicillin G; Lane 3, vaccine extract (5 μl) eq. to 0.25 ml vaccine to which 0.1 unit penicillin G/ml had been added; Lane 4, 0.0125 unit penicillin G.

were necessary in developing a method that is free from interference and is capable of detecting 0.001 unit of penicillin or one part/billion in the finished vaccine. The method involves extraction of penicillin from the vaccine, followed by paper chromatographic analysis of the extract. Concentration of penicillin in the sample is calculated by comparing the bioautographic zones of inhibition produced by the sample with zones produced by penicillin standards.

Methods. Known quantities of penicillin were chromatographed on dry Whatman #1 paper (19 cm $\times$ 46.5 cm) which had been buffered at pH 6.2 with 0.75 M phosphate buffer. During periods of high humidity, more consistency in zone sizes was obtained by applying penicillin standards in a 50% aqueous acetone solution. After evaporation of the solvent used in applying the penicillin sample, the sheet was developed for 16 hours by descending chromatography in a system consisting of diethyl ether saturated with water. At the end of development, the sheet was air dried and placed for 10 to 15 minutes on agar plate at pH 6.5 which had been seeded with *Bacillus subtilus,* ATCC 6633. After removal of the papergram, the plate was incubated 4 hours at 37°C and was left overnight at room temperature. Zones of inhibition due to penicillin, which had diffused from the papergram into the agar plate, were clearly visible and were photographed for a permanent record. Further details concerning the chromatographic and bioautographic procedure may be obtained from Bird and Pugh (2). To extract the penicillin, a sample of 100 ml of vaccine was adjusted to pH 2.2 and

was immediately extracted 3 times with 25 ml aliquots of cold anhydrous diethyl ether, (analytical grade). The ether extracts were withdrawn from the aqueous phase and were combined in presence of 1 ml of 0.1 M phosphate buffer at pH 7. Addition of the buffer at pH 7 insured stability of the penicillin in ether extracts. The procedure described may be carried out in a chillroom or in the laboratory at room temperature if the vaccine is kept cold. Ether-buffer mixture was concentrated under reduced pressure to the buffer phase. The concentrate of ether extractable material was adjusted to 2 ml by addition of acetone. Aliquots of this extract containing 0.03 unit or less of penicillin were chromatographed, following the procedure described for penicillin. The penicillin content of the vaccine was calculated by comparing zone sizes of the unknown samples with zone size of a known quantity of penicillin which had been chromatographed on the same sheet.

Results. A bioautograph which had been obtained from a chromatogram of penicillin is shown in Fig. 1. The antibiotic had been applied in the respective lanes at levels ranging from 0.0025 unit to 0.02 unit. Although a zone of inhibition is observable for 0.0025 unit of penicillin, this minute quantity is not routinely detectable. Consequently, 0.005 unit has been set as the minimum detectable level by the procedure described. If an amount of penicillin standard greater than 0.03 unit is chromatographed, it is difficult to use these zones for estimation of the antibiotic in an unknown sample. Zone sizes obtained at these higher concentrations of penicillin do not show the proportionate differences between concentrations that are observed at lower levels. The most accurate comparison of zone size between the standard and unknown sample is obtained in a range of 0.0075 unit to 0.03 unit of penicillin.

A bioautograph of a chromatogram on which a vaccine extract had been chromatographed is shown in Fig. 2. Since this vaccine contained less than 0.001 unit of penicillin/ml, this concentration of antibiotic was added to the vaccine and an extract prepared and chromatographed as described in the procedure. It is apparent from Fig. 2 that this concentration of penicillin is recoverable and detectable under conditions of this procedure.

It will be observed in Fig. 2 that zones of inhibition occur at the origin of each lane on which the vaccine extract was applied. Although the identity of these inhibitory materials has not been defined, this inhibition is probably due to the preservative, thimersol (Merthiolate), contained in the vaccine and perhaps to minute amounts of dihydrostreptomycin. The zones observed at the origin are not due to penicillin as evidenced by recovery data and also by the effect of penicillinase on the bioautograph. If penicillinase is added to the agar prior to pouring the plate for the bioautograph, the inhibitory material which has migrated from the origin is destroyed; however, the inhibitory material remaining at the origin is unaffected by the enzyme. In both Figs. 2 and 3, the migrating material extracted from the vaccine shows the same R_f value as the penicillin standards. Additional evidence for identifying the migrating material as penicillin is provided by the effect of penicillinase.

The results of another recovery study are shown in Fig. 3. Penicillin was added at a concentration of 0.05 unit and 0.1 unit/ml to 100 ml aliquots of vaccine. Aliquots of the respective vaccine extracts containing equivalent amounts of penicillin were chromatographed adjacent to 2 levels of penicillin. As seen in Fig. 3, excellent recovery of these levels of added penicillin was achived as indicated by similarity in zone sizes.

It has been possible to determine by our method that some lots of finished vaccine contain less than 0.001 unit of penicillin, while other lots contain a concentration of penicillin between 0.001 unit and 0.5 unit/ml.

Summary. A method is described for determining penicillin in poliomyelitis vaccine. This method consists of acidifying the vaccine, extracting the penicillin into diethyl ether, removal of ether by vacuum distillation and transfer of penicillin into a buffer. The penicillin is determined by standard paper chromatographic and bioautographic technics. This method is capable of detecting 0.001 unit of penicillin/ml finished vaccine.

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Some Pharmacological Observations on Tranylcypromine (SKF *trans*-385); A Potent Inhibitor of Monoamine Oxidase.* (25256)

RALPH E. TEDESCHI, DAVID H. TEDESCHI, PETER L. AMES, LEONARD COOK, PAUL A. MATTIS AND EDWIN J. FELLOWS

Smith Kline and French Labs., Phila., Pa.

Zeller *et al.*(1) have demonstrated iproniazid (1-isonicotinyl-2-isopropylhydrazine) to be an inhibitor of monoamine oxidase. Shore and Brodie(2) and Brodie and Shore(3) have reported on the pyrexia, central nervous system (CNS) stimulation and sympathomimetic effects produced in rabbits pretreated with iproniazid followed by reserpine. These effects included increased motor activity and alertness, dilation of the pupil, increased heart rate and piloerection, and were attributed to the action of iproniazid as an *in vivo* inhibitor of monoamine oxidase. We have studied the effects produced by a new and potent *in vivo* inhibitor of monoamine oxidase, (tranylcypromine; 2-phenylcyclopropylamine hydrochloride); and compared its activity with both iproniazid phosphate and *d*-amphetamine sulfate.

Method. The test procedure employed was similar to that described by Brodie and Shore (3), and Horita(4). Several groups of rabbits were pretreated with a range of doses of either iproniazid (25 to 100 mg/kg), tranylcypromine (0.1 to 2 mg/kg), or *d*-amphetamine (1 to 5 mg/kg). Reserpine was administered at various intervals *thereafter* and rectal temperature recorded in an attempt to measure pyrexia at the time of *peak* drug action. The animals were also observed for signs of CNS stimulation. The potency of tranylcypromine relative to that of iproniazid was determined in 3 groups of 6 rabbits each. Groups of rabbits were treated with various doses of either compound. Reserpine was then administered at the time of peak drug action as previously determined. A regression line was obtained by plotting the per cent of rabbits exhibiting rectal temperatures of 42°C or higher against log dose. The dose which caused a rise of rectal temperature of 42°C or higher in 50% of rabbits (ED_{50}) and 95% fiducial limits were determined by the method of Litchfield and Wilcoxon(5). The durations of action of both tranylcypromine and iproniazid were determined by administering ED_{50}s of these compounds to groups of 6 rabbits. This was followed by administration of reserpine at various intervals thereafter. All compounds were investigated in rabbits by the intravenous route of administration. The dose of reserpine employed in all cases was 6 mg/kg. Rectal temperatures were determined with a thermistor thermometer. A converse series of experiments was also performed in which rabbits were first treated with reserpine and 2 hours later, when the animals were deeply sedated, either iproniazid, tranylcypromine or *d*-amphetamine was tested for its ability to arouse the rabbits from their reserpine stupor.

Results. Tranylcypromine produced maximum pyretogenic effects when it was administered 6 hours prior to reserpine. Time of peak effect for iproniazid was 20 hours.

High doses of *d*-amphetamine administered 1 to 6 hours prior to reserpine failed to produce the typical syndrome of symptoms consisting of pyrexia and central excitement. Tranylcypromine was found to be approximately 250 times more potent than iproniazid in this test procedure. The regression lines obtained by plotting the per cent of rabbits

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