The various fractions deriving from the extract of mouse brain secured during the procedure of isolation of PL, AB were tested in mice for their encephalitogenic activity. It was found that the PL, AB, which amounted to 5% of the original constituent solids, was at least as active as whole brain, but the residue obtained after extraction with a chloroform-methanol-water mixture; proteolipide C; and the residue of the chloroform phase, all of which aggregated the remaining 95% of the original solids, were inactive. Thus from the procedure here employed it appeared that the encephalitogenic power of brain tissue, as revealed by inoculation of mice, was possessed only by one of its constituents, namely, PL, AB.

The encephalitogenic activity of PL, AB was evinced only if the protein and lipid constituents remained in chemical combination. If the bond was split, even by the milder procedures, the resultant material was found to be inactive, as were the separated protein and lipid fractions. The possibility remained, however, that the methods of splitting the bond used in this study might have changed the chemical composition of the constituents. Further work on fractionation by other means would therefore appear to be indicated.

Conclusions. Of 4 fractions prepared by chloroform-methanol extraction of mouse brain, only one, proteolipide A and B, is encephalitogenic in mice. Its desiccation which brings about a split in the bond between its protein and lipid constituents results in a loss of its encephalitogenic power and the separated protein and lipid fractions are also inactive.

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Partial Purification of Bovine Intestinal Alkaline Phosphatase.* (19952)

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Hers(1). and Kallman(2) reported that intestinal alkaline phosphatase is concentrated in the microsome fraction of rabbit mucosal cells. We have confirmed this observation, and found that the same is true in the intestinal mucosa of the steer. It is the purpose of this report to demonstrate that the isolation of the microsome fraction may advantageously be made the initial step in the preparation of a soluble preparation, with reproducible properties, from steer mucosa. This method avoids the use of preliminary autolysis or tryptic digestion, heretofore considered essential to the purification procedure, and seems more likely to lead eventually to the isolation of a pure enzyme.

Materials and methods. The bulk separation of steer intestinal microsomes was carried out on material obtained within 30 minutes of slaughtering, kept at 2°C, and was performed as follows: the inside of the duodenum was rinsed with 0.25 M sucrose, and the mucosa stripped from it. The mucosa was suspended in 2 volumes of 0.25 M sucrose, the pH of which had been brought to 8.0 with $KHCO_3$. The suspension was homogenized in a Potter-Elvehjem homogenizer designed to

* Laboratory facilities were provided in part by a gift from Southern California State Dental Association to the School of Dentistry.

handle large quantities of material. The homogenate was made up to a volume equal to 10 times the initial weight of the mucosa by the further addition of sucrose solution. It was then centrifuged at 14,000 × g for 10 minutes in the Spinco preparative ultracentrifuge. This treatment removed the cell fragments, nuclei, and mitochondria. The sediment was resuspended and brought to a volume 5 times the weight of the mucosa, and recentrifuged. The 2 supernatants, which contained the microsomes, were combined. The microsomes were brought down by adjusting the pH of the combined supernatants to 5.0 with 0.1 N acetate buffer, and centrifuging at room temperature after agglutination. This procedure was adopted after it had been found that the results were comparable to those obtained with microsomes isolated by high speed centrifugation from 0.25 M sucrose. The precipitated microsomes were washed twice with distilled water, and finally suspended in a volume of distilled water equal to one-half the original weight of the mucosa. The suspension was placed in a bath at 37 C and one-half volume of n-butanol saturated with water was added slowly with rapid stirring. The rapid stirring was continued for 30 minutes. At the end of this period, the emulsion was broken by centrifugation, and the aqueous layer removed. The butanol layer and the gel, which appeared at the interface of the water and butanol, were reextracted with one-half the original volume of water. The aqueous layers were combined, and lyophilized. Enzymatic activities were determined by the hydrolysis of p-nitrophenylphosphate, at pH 9.1 and 37° C. The buffer used was 0.1 M bicarbonate-carbonate, containing 0.001 M magnesium acetate. Protein determinations were performed with Lowry's modification of the Folin-Ciocalteau reagent(3). All protein values are referred to an albumin standard. The solubility of the soluble material obtained from the butanol treated microsomes was studied in acetone-water mixtures at 2° C. A 2% solution of the lyophilized material was made in water at 2° C. The solution was allowed to stand for several hours at this temperature, and then centrifuged. Any insoluble material was removed, and the supernatant used for the precipitation studies. Two ml of the solution were used for each determination. Sufficient water and acetone were added to obtain the desired acetone concentration in 8.0 ml of solution. Protein concentration and enzymatic activity were determined on the supernatants after removal of the precipitated material. Filter paper electrophoresis studies were made on the 2% aqueous solution prepared for the solubility studies, and were carried out at pH 8.6 (0.05 M veronal) and pH 6.9 (0.05 M imidazole). The procedure was essentially the same as that described by Kunkel and Tiselius (4). The protein was detected by its color reaction with 0.1% ninhydrin in water-saturated butanol. A second, parallel strip, was used to determine the position of the enzyme. This strip was cut into 1 cm pieces, and the enzyme was eluted from each piece and the activity determined.

Results and discussion. In our investigation of methods for the purification of intestinal alkaline phosphatase the procedure initially followed was that of Albers and Albers (5). The crude enzyme was isolated from an autolysate of steer mucosa, and then subjected to fractional precipitation with acetone in the cold. It was thought that by suitable variation of the conditions of precipitation and extraction, the protein impurities might be removed. The results were unsatisfactory, since the impurities appeared to have solubility characteristics which were virtually identical with those of the enzyme. Filter paper electrophoresis of the various fractions at pH 8.6 yielded, in each case, a single long streak of anionic protein with no well-defined

TABLE I. Alkaline Phosphatase in Homogenate and in Soluble Fraction from Butanol Treated Microsomes.

Preparation	Specific activity* Homogenate	Specific activity* Soluble fraction from microsomes	Purification†
1	4.55	115	25
2	8	118	15
3	5.4	128	24

* Expressed as mM of p-nitrophenylphosphate split/hr/mg protein.

† Ratio of specific activity of soluble fraction from microsomes to that of the homogenate.

peaks. The enzyme presented a similar picture superimposable upon the protein.

Separation of the microsome component of the cell from the rest of the cellular material results in a 3- to 5-fold increase in the specific activity of intestinal alkaline phosphatase. Although this increase is not great, 70 to 80% of the extraneous cellular protein has been removed, leaving a system which is better defined than either an homogenate or an autolysate of mucosa.

The butanol extraction, similar to that used by Morton(6), and Zittle(7), was selected because of their success in obtaining soluble phosphatase preparations, and because it might be expected to disrupt a lipid-containing complex such as the microsome. The extraction results in a clear, aqueous solution of alkaline phosphatase which is not sedimentable by centrifuging for 2 hours at 20,000 × g. Denaturation of some of the other proteins in the microsomes during the butanol treatment probably contributes to the purification of the enzyme despite the fact that some loss of enzyme also occurs and the recovery at this step is approximately 50%. The purification achieved is variable, and seems to be inversely related to the activity of the initial homogenate, since the final activity of each of the 3 preparations studied is approximately the same (Table I).

Despite the considerable degree of purification obtained, the material from butanol-treated microsomes still contains a considerable amount of inert material. The amounts of protein and enzymatically active material precipitated at various acetone concentrations are shown in Fig. 1. Although the bulk of the enzyme is precipitated between 40 and 50% acetone, little protein precipitates until the acetone concentration is above 50%. Indeed, the amount of protein precipitated between 40 and 50% acetone was too small to be measured accurately as the experiments were carried out. From the amount precipitated above 50% acetone, it has been estimated that the amount of protein precipitating with the enzyme could not be more than 8-10% of the protein present.

The same conclusion is indicated by the electrophoretic results. Fig. 2 shows the distribution of the enzyme after electrophoresis for 16 hours at 5.7 volt/cm, pH 8.6. The positions of several distinct protein peaks are shown, and it will be seen that the enzyme is not associated with any of these. From an evaluation of the strips developed for protein, it can be concluded that at least 90-95% of the protein is material other than alkaline phosphatase. The results obtained at pH 6.9 are similar.

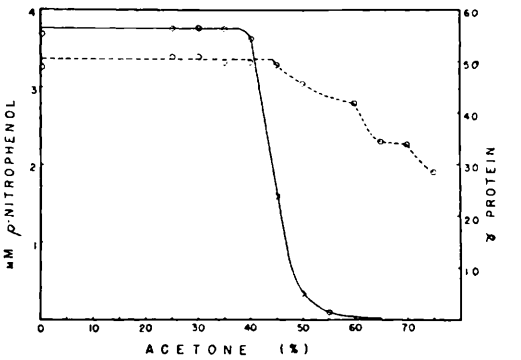

FIG. 1. Comparison of solubility of enzyme (solid line) and protein (dashed line) at increasing concentrations of acetone, in the soluble fraction of microsomes after butyl alcohol treatment. Enzyme activity at left represents mM of p-nitrophenyl phosphate split, under identical conditions, by aliquots of supernatant from the precipitation at each acetone concentration. Protein remaining in supernatant is indicated on scale at the right.

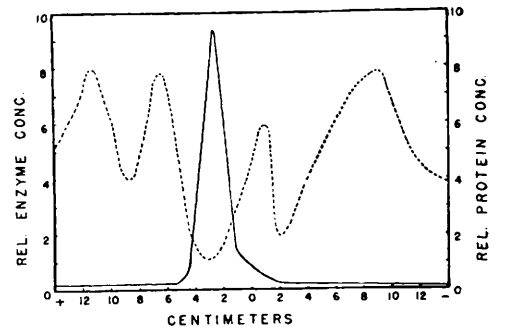

FIG. 2. Results obtained on subjecting the soluble fraction from butanol treated microsomes to electrophoresis in filter paper at pH 8.6. Distance of migration is shown at bottom. Relative concentration of protein is indicated by dashed line, the scale in arbitrary units on right. Enzymatic activity is shown by solid line, the scale in arbitrary units on left.

Summary. A procedure has been presented for obtaining a soluble preparation of intestinal alkaline phosphatase from the microsomes of steer intestine mucosa by extraction

of the microsomes with n-butanol. Although the purification achieved is only 15 to 25 times that of the original homogenate of the tissue, the resulting preparation appears to be particularly suitable for further purification by fractional precipitation with acetone.

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Duration of Drug Action. (19953)

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A drug effect has two major features, 1) *intensity* and 2) *duration*. Intensity of drug effect is unquestionably a variable magnitude, but its quantitation meets considerable obstacles because its dimension is difficult to define. The assumption, followed in some cases, that intensity of effect can adequately be measured in such simple dimensions as length (muscle shortening), volume (glandular excretion), weight, etc., is certainly fallacious; in other cases, *e.g.*, drug-produced convulsions, one is at a loss to quantitate in any physical dimension. Yet the need to analyze the relationship between intensity of effect and its independent variable, *drug dose*, and to compare intensities of effect of different drugs has led to the elaboration of a workable yardstick for intensity of effect. This was possible by circumventing the problem of the physical dimension and focusing attention to the biological dimension. The biological yardstick is "notched" in terms of *endpoints, i.e.*, selected effects of well specified intensity, such as: *threshold effect; peak effect* = greatest intensity attained in the course of the action of a dose; *maximal effect* = greatest intensity obtainable with any dose; and numerous intermediate endpoints of appropriate specification. This instrument, refined by an abundant armamentarium of biostatistical technics to overcome variability, has placed evaluation of intensity of effect on a satisfactory conceptual and procedural basis.

The concepts and principles for quantitation of the other feature of drug effect, namely, the *duration*, are as yet on an incomparably lower level of development. This may seem paradoxical because, at first thought, the problem of dimension does not appear to offer any difficulties; by definition, the temporal features of drug effect present themselves in the physical dimension of time. Nevertheless the concept of *duration of effect* has remained rather vague and hence of little constructive value. The reasons become obvious when the intensity and time characteristics of the effect of a drug dose are represented graphically (Fig. 1). Such a diagram points out that, quite similar to the intensity axis, the temporal axis of effect is also subdivided into biological "endpoints". Since evidently the temporal features are correlated with intensity, the temporal endpoints are connotatively coupled to those of intensity. As exemplified in the diagram for action III, the temporal endpoints are time intervals measured from the time of drug administration, T_o, to that of an endpoint of intensity of effect. Major temporal endpoints are: *latency time* = time of onset of threshold effect (T_a); *peak time* = time of (onset of) peak effect (T_1); *persistence time* = time of disappearance of threshold