2.0 mg—120 ± 15% above preinjection values—5 preparations). Time courses of bile flow after these two agents in typical experiments are shown in Figs. 1 and 2.

In a series of 16 preparations involving 35 injections, the total solid contents of bile before and after choleretics were determined on samples collected at 10-min intervals. Ten of these experiments involved the administration of dehydrocholate only (20 injections), 3 of bile salts only (5 injections), and 3 of bile salts and dehydrocholate administered in succession to the same preparation (5 injections of bile salts, 5 of decholin). Typical results of the experiments are represented in Figs. 1 and 2: choleresis induced by

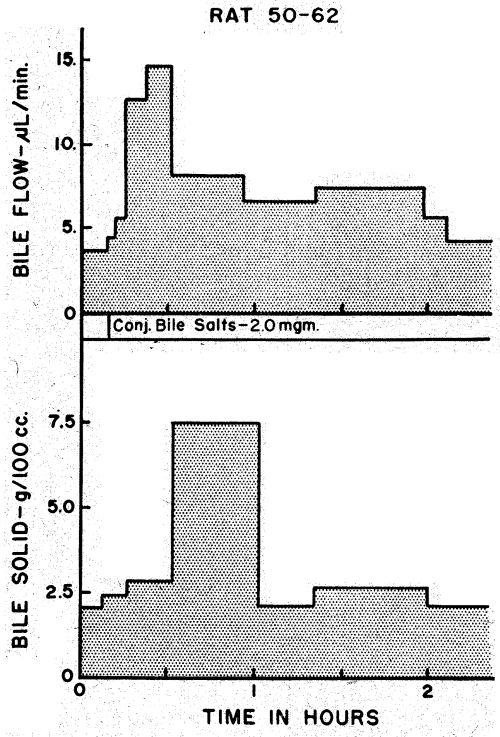

FIG. 2. Bile flow and bile solids after conjugated bile salts. Rat liver perfusion 50-62, ♀, 291 g, 0 time—240 min perfusion. Perfusate flow = 66 ± 3 ml/min.

dehydrocholate was accompanied by unchanged total solid concentration in 17 injections, and by decreased total solids in 8 injections; choleresis induced by conjugated bile salts was accompanied by pronounced increases in the total solid content of the bile in 9 out of 10 injections.

Perfusate flow through the liver, determined at 2-min intervals, showed no consistent changes following injections of either drug. The method employed was such that 10% changes in flow would have been detected.[3]

In the isolated liver preparation choleretic as well as hydrocholeretic effects can be elicited. In fact, the preparation responds more markedly than the intact animal, possibly as a result of bile salt depletion in the isolated circulation.

The parallel responses of isolated, perfused livers and of livers in intact animals justify application of the present results to the question raised originally. The isolated liver is perfused through the portal vein only; thus, changes in hepatic arterial blood flow cannot possibly occur. Since hydrocholeresis and choleresis are nonetheless clearly distinguishable in this preparation, a specific vascular response cannot be the basis of the difference in action between conjugated bile salts and dehydrocholic acid.

Again, total blood flow also remained unchanged in these experiments. Recalling that the blood supplied to the liver *in vitro* is saturated with oxygen—differing from that supplied in the intact animal—these facts lead to the following conclusion: If sufficient oxygen is supplied to the liver, choleresis as well as hydrocholeresis can be observed without accompanying changes in blood flow. Hence, differences between the two types of drug action depend upon some direct effect on the hepatic parenchyma, most likely on the hepatic cells.

[3] This has since been confirmed by using a continuously recording flowmeter, sensitive to fluctuations in flow of ± 1%.

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The Purity of Crystalline Lactic Dehydrogenase

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In view of the results obtained by Theorell (*1, 2*) on the kinetics and mechanism of action of alcohol dehydrogenase, it seemed of interest to investigate lactic dehydrogenase in the same manner.

The lactic dehydrogenase investigated in the present study was Straub's (*3*) crystalline enzyme from heart muscle. Several preparations were made, and all gave the electrophoretic pattern shown in Fig. 1. This observation is the subject of the present communication.

In Fig. 1 two components are shown migrating to the anode, the major component having the greater velocity. Experiments in the pH range of 5–7 showed the two components to have a mobility difference of -0.8×10^{-5} cm^2 v^{-1} sec^{-1}. Electrophoresis was therefore used to obtain pure samples of each component. In 0.5 saturated ammonium sulfate solution the pure major component yielded crystals that appeared

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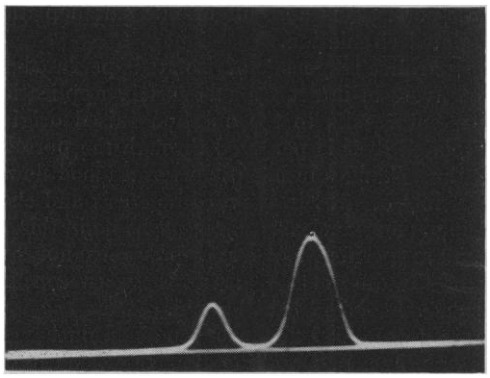

FIG. 1. The electrophoretic pattern of crystalline lactic dehydrogenase. Descending pattern in phosphate buffer pH 5.7, ionic strength 0.1. The salt boundary is just visible as a thickening of the base line on the extreme left.

identical with those in the unresolved enzyme or the crystals shown by Straub (*3*). This material migrated as a single component on electrophoresis. So far it has not been possible to crystallize the minor component.

The salting-out diagram, optical density at 280 mμ vs percentage saturation with ammonium sulfate, also shows a two-component system. The breaks in the salting-out curve are found at 40 and 43% ammonium sulfate saturation.

The activity of the enzyme was determined by measuring the rate of appearance of the absorption band of DPNH at 340 mμ. The tests were carried out in glycine-NaOH buffer, pH 9.6, with excess of DPN (*4*) and lactate. Both components were found to oxidize L(+) sodium lactate. The major component was usually somewhat more active on the basis of optical density at 280 mμ. D(−) sodium lactate was not attacked.

It seems to be an interesting fact that following the application of a rigorous test "pure" enzymes, like crystalline proteins, can often be shown to be heterogeneous. Different workers, using a variety of techniques, have provided evidence for different forms of pepsin (*5*), cytochrome *c* (*6*), ribonuclease (*7, 8*) and lysozyme (*9*). The study of such substances should aid in the elucidation of enzymatic reaction mechanisms, regardless of whether the different forms are present in nature.

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The Application of Electrophoresis-Convection in the Purification of Enzymes: Purification of the Alkaline Phosphatase of Swine Kidneys

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The separation of protein mixtures by electrophoresis-convection (*1*) has been quite successful, indicating that the application of this technique to the purification of enzymes should be explored. This note presents initial results of such a study in which a partially purified alkaline phosphatase preparation was the starting material.

The preparation of the alkaline phosphatase from swine kidneys and the determination of the enzyme activity were carried out as previously described (*2*). It was necessary to activate the enzyme before it was assayed. To do this, the final enzyme dilution was prepared in 0.01 *M* DL-alanine which was also 0.05 *M* in $NaHCO_3$-Na_2CO_3 buffer of a pH of 9.1. The diluted enzyme solution was incubated for 3 hr at 25° C to activate it and then assayed for enzyme activity. Total nitrogen determinations were made by the micro-Kjeldahl procedure.

Separations by electrophoresis-convection were carried out at 5° C, using a 0.1 ionic strength phosphate buffer at pH 7.2. The duration of the runs was 6 hr, and the field strength employed was 1.6 v/cm. When necessary, the protein solutions were concentrated by precipitation with $(NH_4)_2SO_4$ or acetone or by lyophilization, followed by dialysis of the enzyme against the phosphate buffer. This same phosphate buffer was used in the electrophoretic analyses.[2]

Table 1 presents the results obtained for a single-stage separation and in addition the final results for

TABLE 1

PURIFICATION OF SWINE KIDNEY ALKALINE PHOSPHATASE BY MEANS OF ELECTROPHORESIS-CONVECTION

Experiment	TPKU*	PU/mg TN†
1. Initial material	226	146
a. Fourth stage		
Top cut	12	392
Bottom cut	29	85
2. Initial material	250	191
a. First stage		
Top cut	95	270
Bottom cut	138	159
b. Fifth stage		
Top cut	15	588
Bottom cut	17	85

* TPKU = total phosphatase units × 10^{-3}.
† PU/mg TN = phosphatase units/mg total nitrogen.

[1] With the technical assistance of Eleanore D. Goodman.
[2] The electrophoretic analyses and mobility calculations were carried out by J. M. Vandenbelt and R. B. Scott, of Parke, Davis & Co. Their generous assistance is gratefully acknowledged.