folic acid by utilizing simple precursors, as evidenced by its ability to grow on a defined medium consisting of glucose, ammonium nitrate, salts, thiamine, biotin and agar. Although sulfa drugs can inhibit growth, inhibition is reversed by p-aminobenzoic acid, indicating that the sulfa drugs interfere in folate biosynthesis. No potentiation of this bioactivity was observed in the presence of trimethoprim, which itself lacked anticandidal activity. In contrast, aminopterin and methotrexate, also inhibitors of bacterial DHFR, were active against *C. albicans.* Moreover, these compounds did show synergy with the sulfa drugs.

Since the lack of activity of trimethoprim could be due to permeability barriers, subinhibitory concentrations of amphotericin B (0.5 µg/ml) or econazole (0.1 µg/ml) were incorporated into the agar to increase the cellular permeability[7,8]. Trimethoprim was still without effect. Also, the activities of aminopterin and methotrexate were not noticeably enhanced by the permeabilizing agents. These data suggest that penetration into the cell is not a limiting factor for either the active compounds or trimethoprim, a compound of approximately 0.6 the molecular weight of the other 2 antifolates.

The affinity of trimethoprim for DHFR was then studied. As shown in the table, trimethoprim has poor affinity for the candidal and mammalian enzymes, in contrast to the enzyme from *Escherichia coli.* Aminopterin and methotrexate, on the other hand, act as tight-binding inhibitors in all the systems tested. These data, therefore, are sufficient to explain the lack of trimethoprim activity against *C. albicans.*

Because of the resemblance to mammalian enzyme, *C. albicans* DHFR is not an appealing target for chemotherapeutic purposes. However, the metabolic steps involved in folate biosynthesis may be exploitable in seeking an anticandidal agent.

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The application of sodium deoxycholate and Sephacryl-200 for the delipidation and separation of high density lipoprotein

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Summary. A method to remove lipids from human plasma high-density lipoprotein (HDL) was developed. The procedure required column chromatography on Sephacryl-200 in the presence of the bile salt sodium deoxycholate. The lipid free protein obtained retained the immunological properties of the native HDL.

Previous procedures for preparing soluble apo-HDL depend on delipidation of HDL by extraction with organic solvents[2]. The methods led to incomplete recovery of the apo-protein because some of the polypeptide chains of HDL were preferentially solubilized in the organic solvents[3]. Studies on the effects of detergents on low-density lipoproteins (LDL) showed that 4 detergents could displace all the lipid from LDL and the soluble apo-LDL could be isolated by gel filtration[4]. Best results were obtained with the bile salt sodium deoxycholate (NaDOC)[4]. With NaDOC the apo-LDL did not undergo conformational changes, and the detergent could be removed completely from the apo-protein[4].

In connection with the work in this laboratory on the

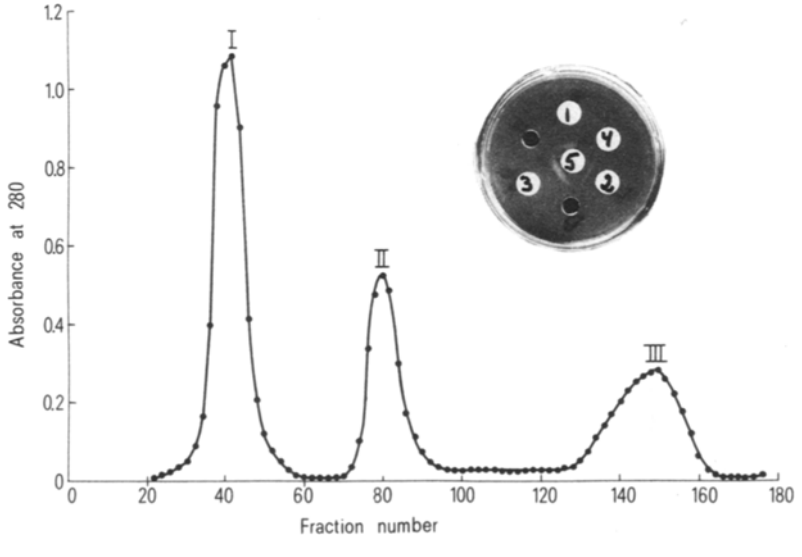

Figure 1. Elution profile of human plasma lipoprotein. Lipoproteins quantitatively removed by ultracentrifugation of plasma at d1.225 were applied to a Bio-Gel A-5 m agarose column 2.5 cm × 90 cm, and eluted in 0.15 M NaCl-0.01% EDTA at 12 ml/h, 2 ml/fraction at 10 °C. Immunodiffusion of various HDL preparations. 1. Native HDL; 2. apo-A; 3. top fractions of protein peaks III; 4. top fraction of protein peak I or II; 5. anti-Apo A.

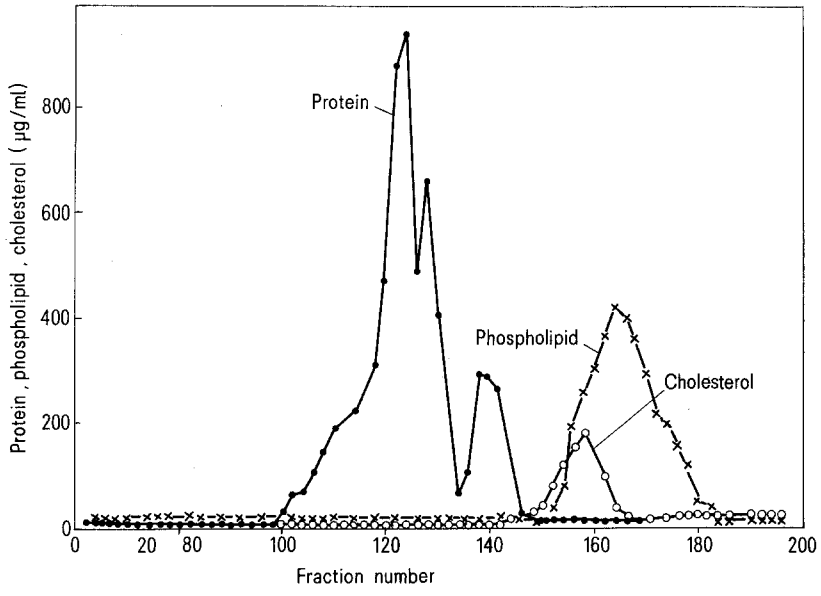

Figure 2. Separation of apo-HDL and HDL lipids in the presence of NaDOC. Column: Sephacryl-200 (2.5×90 cm). Buffer: 10 mM NaDOC–0.05 M NaCl–0.05 M sodium carbonate (pH-10). Sample 35 mg HDL in 7 ml of 0.05 M NaCl–0.05 M sodium carbonate (pH-10) with 420 mg NaDOC added. Elution rate was 40 ml/h, fractions 3 ml. ●, Protein, ○, cholesterol, ×, phospholipids.

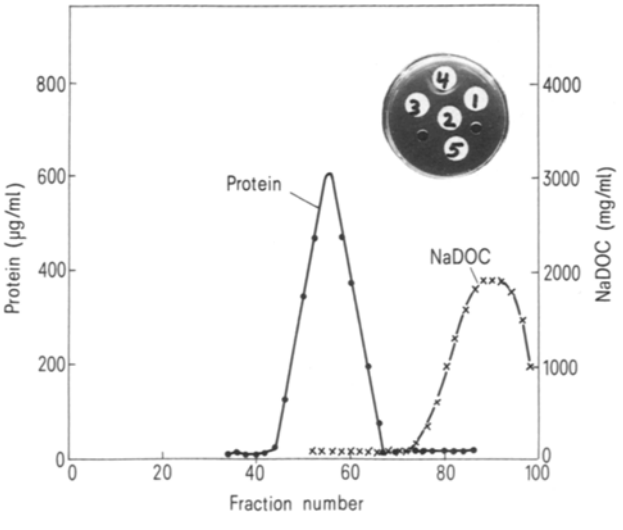

Figure 3. Removal of NaDOC from apo-HDL preparations. Column: Sephadex G-50 (2.5×45 cm) Buffer: 0.1 M Tris (pH 9.0). Sample: 1.5 mg apo-HDL in 3.5 ml of 10 mM NaDOC, 0.05 M NaCl and 0.05 M sodium carbonate (pH 10). Flow rate 15 ml/h, fractions 2 ml. ●, Protein, and ×, NaDOC. Immunodiffusion of purified apo-HDL: 1. apo A; 2. apo-HDL purified with NaDOC (top fraction of protein peak I); 3. Native HDL; 4. anti-HDL serum; 5. anti-apo B serum.

determination of HDL in human serum, large quantities of apo-HDL were required. In the initial stages of this study established methods[2,5] for the preparation of apo-HDL were followed. In the present experiments the application of NaDOC and gel chromatography was found to be simpler and less time consuming than sequential centrifugation and delipidation with organic solvents[2,5]. The apo-HDL obtained showed immunological properties comparable with native HDL.

Materials and methods. Human blood (60 ml) was collected and mixed with EDTA (final concentration 2.6 mM). Plasma was isolated by centrifugation at 1000×g at 15 °C for 15 min.

High density lipoprotein (HDL) was isolated from plasma by a method described by Rudel et al.[6], with some modifications. Solid KBr (0.317 g of KBr/ml plasma) was added to raise the density of the plasma to 1.225. Plasma (d1.225) was then placed into 6.35×1.27 cm diameter cellulose nitrate centrifuge tubes (4 ml/tube) and overlayered with 2 ml d1.225 solution which was prepared by addition of solid KBr to the buffered d1.006 solution of Scanu and Granda[7]. Tubes were centrifuged in a 50.3 Ti fixed angle rotor (Beckman) for 24 h at 15 °C and 40,000 rpm in a Beckman L5-75B ultracentrifuge. The centrifuge was stopped with the brake off, and the tubes were carefully removed from the rotor and the top 1.5 ml containing the lipoprotein concentrate was removed with a Pasteur pipette. About 6 ml of the lipoprotein was applied to an agarose chromatography column. The jacketed column (25 cm×90 cm) was packed with Bio-Gel A-5 m 200–400 mesh as recommended by the manufacturer (Bio-Rad Laboratories, Richmond, California) fitted with flow adaptors and cooled to 10 °C with water. The column was eluted with 0.15 M NaCl–0.01% EDTA pH 7.0 buffer in an upward direction with the aid of a peristaltic pump and the protein elution was monitored with a dual path UV-monitor (Pharmacia, Canada Ltd).

The isolated peak which reacted only with anti Apo A by double immunodiffusion was considered to be analogous to HDL. The HDL segment of the elution profile was dialyzed overnight against 0.05 M NaCl–0.5 M sodium carbonate (pH 10) at 4 °C. The detergent NaDOC (Fisher Scientific) was added to the HDL solution (final concentration 12 mg NaDOC/mg HDL) and applied to a column (2.5×90 cm) fitted with flow adapters and packed with Sephacryl-200 (Pharmacia). Packing and equilibration of Sephacryl-200 were done as recommended by the manufacturer. The column was run upward with the aid of a peristaltic pump (LKB) at room temperature.

Protein was determined by the method of Lowry[8] using bovine albumin (Sigma) as a standard. Phosphorous was measured directly from the eluent fractions according to Bartlett[9]. Total cholesterol was determined enzymatically[10] using the Abbott cholesterol kit. The bile salt NaDOC was determined as described[4].

Results and discussion. The elution profile of plasma lipoproteins isolated from humans is shown in figure 1. A separation into 3 classes of lipoproteins was obtained. The column separation is on the basis of size[6], the largest lipoprotein fraction eluted first, VLD, followed by LDL and HDL. Double immunodiffusion used to identify HDL

shows (fig. 1) that only peak 3, fractions 128–160, reacted only with anti Apo A and fused with apo A and native HDL. The HDL peak was concentrated in an Amicon filter and chromatographed on Sephacryl-200 in the presence of NaDOC. Figure 2 shows the presence of 3 protein peaks between fractions 100 and 146. The protein fractions contained no detectable phospholipids or cholesterol. The phospholipids and cholesterol eluted between fractions 150 and 184 and no protein was detected in the eluant. If lower concentration of NaDOC (< 10 mg/mg lipoprotein) were added some of the cholesterol esters and triglycerides eluted with the protein. The concentration of bile salt and NaDOC used in these experiments removed all detectable lipids from HDL. Bile salts are known to form small mixed micelles with lipids such as cholesterol[11], phospholipids[12] and glycerides[13], which can be removed by gel filtration[4].

Resolution of apo-HDL into 3 components (fig. 2) suggests that the polypeptide constituents of HDL have separated. Studies on the characterization and distribution of apolipoproteins have shown that the major density classes are heterogeneous not only with respect to particle size but also with respect to apolipoprotein composition[14–16]. Apo-HDL contains several distinct apoproteins namely A-1, A-11, C-1, C-11, C-111, D[17]. The present studies indicate that the detergent sodium deoxycholate may aid in the resolution of the apoproteins of apo-HDL. Similar resolution of the polypeptide of apo-HDL with another detergent, sodium dodecyl sulfate, was demonstrated previously[15]. The use of Sephacryl-200 instead of Sephadex G-200 commonly employed for the separation of apoproteins[4,18] improved the separation of apo-HDL from HDL lipids and increased the flow rate 5-fold.

The bile salt was removed by gel filtration on a Sephadex G-50 in a detergent-free medium (fig. 3). When the eluted protein was tested against anti-HDL serum the lipid-free apo-HDL gave a precipitin line which fused with that given by native HDL and apo-A (fig. 3). No precipitin lines were formed when the apo-HDL was tested against anti apo-B serum.

In the procedures described, HDL and apo-HDL were obtained by gel chromatography. The detergent NaDOC formed small mixed micelles with the lipids[11] which were separated on Sephacryl-200 packed columns; in addition, longer columns were necessary to obtain satisfactory separation. The apo-HDL obtained gave one precipitin line with anti-HDL which fused with the line obtained with native HDL. These qualitative studies indicate that the apo-HDL carries all the major immunological determinants of the intact molecule of HDL, and that the apo-HDL has not undergone conformational changes resulting in detectable immunochemical alterations.

1 The author is grateful to Ms L. Gray for her excellent technical assistance and Dr M. Fainaru of the Hadassah Medical Organization, Jerusalem, and Dr C. Heuck of the Klinisches Institut für Herzinfarktforschung of Heidelberg, for kindly supplying purified apo-A, apo-A antiserum and apo-B antiserum.

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Modulation of an 'equilibrium enzyme': Ecological evidence

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Summary. The activity of D-lactate dehydrogenase in the foot of the snail, *Helix pomatia*, is closely correlated with time of year and mean daily temperature, and increases strikingly after the animals have been exposed to a nitrogen atmosphere for 24 h.

Within a given tissue, differences in enzyme activity are usually considered to be 'adaptive' only if they concern non-equilibrium reactions and thus, by definition, regulatory enzymes[2,3]. According to this philosophy, lactate dehydrogenase, an enzyme catalyzing an equilibrium reaction, could not be a candidate for supplying information on enzymic mechanisms of metabolic control. Rolleston[4] has emphasized this point quite recently by stating that the kinetic properties of LDH have nothing to do with aspects of metabolic control but are 'accidents of evolution'. However, despite this warning note, LDH and other enzymes, supposed to catalyze equilibrium reactions, have often been used as indicators of adaptive features of metabolism, particularly in poikilothermic organisms[5,6]. This controversy contains aspects which are of general importance for an understanding of the ways the metabolism of organisms responds to external or internal stimuli. It is also clear that there is a lack of quantitative data on which theories can be built.

I report on measurements of the activities of D-lactate dehydrogenase (D-LDH; EC 1.1.1.28) and pyruvate kinase (PK; EC 2.7.1.40) in the foot of the terrestrial snail, *Helix pomatia*. These measurements were carried out between 1977 and 1980 on animals derived from a single locality (Mühlau) near Innsbruck. The enzymes were extracted from the tissues with an efficiency of about 95%[7], and all activities measured at a temperature of 20 °C. Other specimens were exposed to nitrogen (99.99% purity) for 24 h