and fatty acid for the dystrophic animal was the percentage obtained after comparison with values of the "normal littermate". These results are in Table I and represent the results obtained from 22 pairs of dystrophic mice and their corresponding littermates. The percentage standard deviation of the mean observed throughout these experiments was $\pm$ 21 %. The observed ratios are thus statistically significant except for muscle.

From Table I it can be concluded that homogenized and fortified tissues of dystrophic mice have a greater lipogenesis and cholesterolgenesis than their "normal" littermates. The brain shows the greatest difference, and is followed closely by the liver and kidney. The spleen and skin showed smaller increments. All values presented have been corrected to equal concentrations of nitrogen present in the original homogenate.

These results suggest that definite metabolic differences occur in the various fat-metabolizing centers of the dystrophic mouse.

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Direct ion-exchange chromatography of tissue extracts without precipitation of protein by use of non-ionic detergents

In general, the separation of constituents of a biological preparation by ion-exchange chromatography is done with protein-free filtrates because protein adheres to ion-exchange resins and clogs the columns. In the course of other experimental work, one of us (J.E.S.) chanced to discover that proteins in the presence of certain polyoxyethylene non-ionic detergents did not adhere to ion-exchange resins but flowed through resin columns directly. Small ionized molecules present in such protein-detergent mixtures did not seem to be affected and were retained by the resin column from which they could be afterward displaced sequentially in the usual manner, thereby permitting direct chromatography of tissue extracts.

Abbreviations: OPE_x, *p-t,t*-octylphenoxy-polyoxyethylene-ethanol of x ethylene oxide units; MDTA, methyldodecylbenzyltrimethyl-ammonium chloride; UMP, uridine monophosphate; TPN, triphosphopyridine nucleotide; IMP, inosine monophosphate; AMP, adenosine monophosphate; GMP, guanosine monophosphate; UDP, uridine diphosphate; ADP, adenosine diphosphate; UTP, uridine triphosphate; ATP, adenosine triphosphate; UDPG, uridine diphosphoglucose; PNA, pentose nucleic acid.

The detergents used were a series of *p-t,t*-octylphenoxypolyoxyethylene-ethanols containing 9 to 16 ethylene oxide units per molecule (obtained from Rohm and Haas, Inc., Philadelphia) and some thioether analogs (obtained from Monsanto Chemical Company, St. Louis). Polymer species of specific chain length were obtained by chromatographic separation on silica columns[1]. Detergent concentrations of 0.05 % or more were required; these concentrations are somewhat greater than the critical concentration of detergent at which micelles are formed[2]. For routine chromatography of tissue extracts, crude fractions containing a mixture of several adjacent polymer species were found to be adequate*.

Excised rat tissue, frozen in liquid nitrogen and powdered, was added to 9 vol. 3 % ice-cold detergent solution and mixed thoroughly in a Waring Blendor. Enzyme activity continued in tissue preparations containing OPE detergent alone leading to changes in the constituents. To minimize enzymic alteration, about 0.3 % of a cationic detergent, methyldodecyl-benzyl-trimethylammonium chloride (obtained from Rohm and Haas, Inc., Philadelphia), or 4–8 *M* urea was added. An insoluble residue containing about 20 % of the total protein remained. The amount of insoluble protein and structural elements could be lessened by the use of urea in the homogenizing medium in addition to detergent. At times, the chromatographic columns were clogged by cell particulates. This was avoided by filtration through a coarse fritted glass disc or by sonication of the homogenate and centrifugation at 14,000 $\times$ *g* for 16 min at 0° prior to chromatography. For comparison, $HClO_4$ extracts were prepared according to the directions of HURLBERT *et al.*[3].

A typical direct chromatogram of the free nucleotides of a rat-liver homogenate is shown in Fig. 1, together with a chromatogram of the acid-soluble fraction of a rat-liver homogenate obtained after $HClO_4$ precipitation of protein[3]. While the positions of the nucleotide peaks in the two chromatograms correspond, the total quantity of nucleotides obtained in the direct chromatogram was much less than was obtained with the $HClO_4$ extract. Probably this was due to liberation of nucleotides bound to protein by $HClO_4$ while the detergent did not rupture these protein complexes. This assumption was supported by the observation that additional free nucleotides were found upon chromatographing the $HClO_4$-treated effluent of a direct chromatogram. Though certain protein complexes were broken by the detergent, it often provided a means of separating protein complexes from free ligand. For example, detergent and ion-exchange resins have been used for separating protein-bound iodine from inorganic iodide in the determination of iodine bound to serum protein[4].

The resolution of peaks in direct chromatography is less sharp than in chromatography of protein-free filtrates. The loss of resolution seems to be associated with the presence of protein, since addition of detergent and urea to protein-free filtrates resulted in chromatograms identical in all respects to those obtained in the absence of detergent. Furthermore, fractions obtained from direct chromatograms (which contained neither detergent nor protein) showed the same resolution and separation of component peaks upon rechromatography as fractions from $HClO_4$ filtrates.

Direct chromatograms using detergent of high-speed supernatant fractions of rat liver showed only minor differences in the free nucleotide pattern when compared

* Two useful crude fractions (and their average ethylene oxide chain length) obtained from Rohm and Haas, Inc., Philadelphia, were: Triton X-100 = OPE_{av-10} and Triton X-165 = OPE_{av-16}.

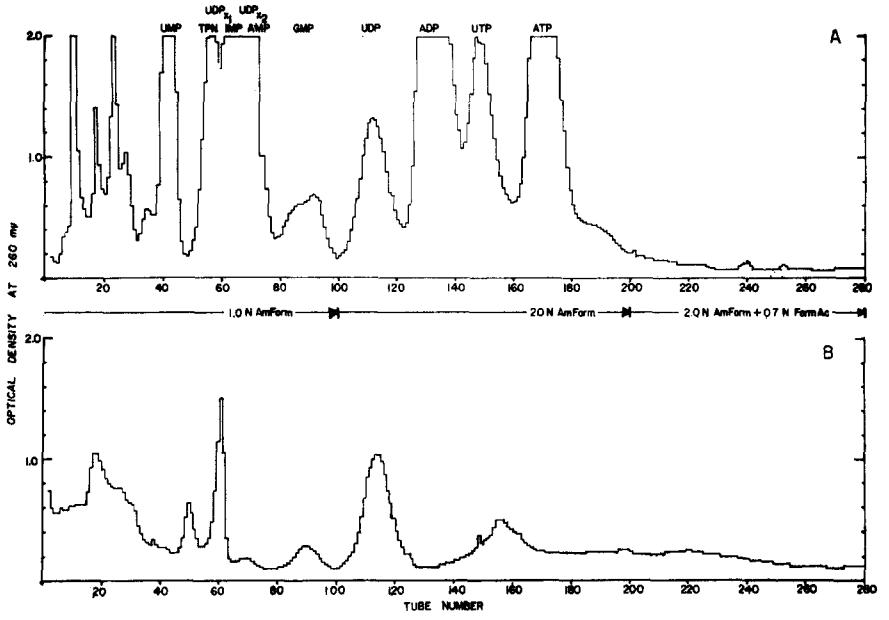

Fig. 1. (A) Chromatogram of $HClO_4$: filtrate of 15 g rat liver on Dowex 1-X10, 200–400 mesh resin gradient eluted with an ammonium formate system at pH 5.0. (B) A similar direct chromatogram of rat liver homogenized in 9 parts 3.0% OPE_{11}, 0.3% MDTA in 0.35 *M* sucrose.

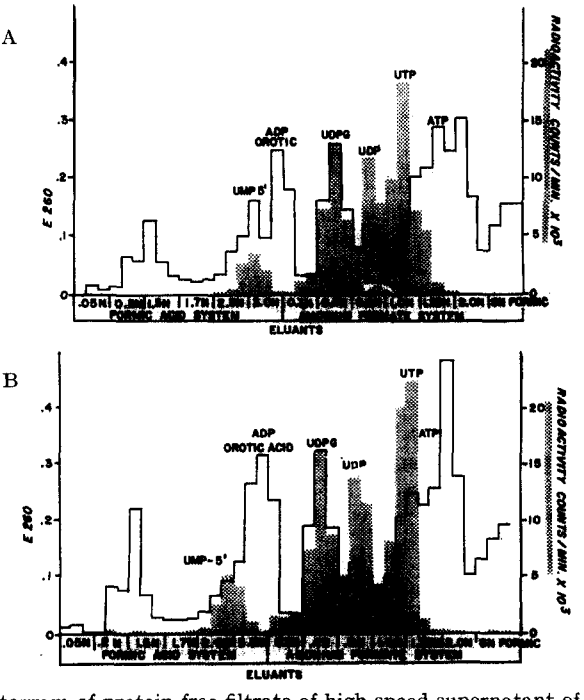

Fig. 2. (A) Chromatogram of protein-free filtrate of high-speed supernatant of rat liver incubated for 90 min with [^{14}C]orotic acid at 37°. (B) A similar direct chromatogram of high-speed supernatant using 0.5% OPE_{11} final concentration after identical incubation with [^{14}C]orotic acid.

with chromatograms of $HClO_4$ filtrates (Fig. 2.) Direct chromatography was particularly useful for the separation of unstable phosphate esters such as phosphocreatine and phosphoglycocyamine; gradient elution with an ammonium formate system at pH 7.2 was used.

No evidence for an interaction between the detergent and ion-exchange resins was found. Titration curves of Dowex 1-X8, 100 to 200 mesh resin in the hydroxyl cycle suspended in water gave an ion-binding capacity of 3.06 mequiv./g wet wt. resin when titrated with HCl and 3.16 mequiv./g in 3.0 % OPE_{11}. In order to evaluate the behavior of this resin with ionic aggregates, it was titrated with *p*-dodecylbenzenesulfonic acid (obtained from the Continental Oil Co., Chicago), a strongly acidic detergent which forms micelles in water. Titration in water gave an apparent ion-binding capacity of 1.98 mequiv./g; in the presence of 3.0 % OPE_{11}, the apparent resin capacity was 1.94 mequiv./g. The differences observed were not considered significant.

A change in specific resin volume often is observed when an ion-exchange resin is put into a medium with which it reacts. No change in resin volume was observed when Dowex 1 or Dowex 50 previously suspended in water was transferred to a medium containing 3.0 % OPE_{11}. A study of the protein interaction is in progress and the results will be the subject of a future communication.

Direct chromatography using OPE detergents appears to be a technique suited for the separation of labile constituents of tissue preparations requiring mild treatment to prevent decomposition. The method is applicable to all substances of low molecular weight that can be separated by ion-exchange chromatography. Protein-bound complexes are likely to remain intact and the ligand not removed by the ion-exchange resin in the presence of detergent.

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