BBA 65568

ELECTROPHORETIC BEHAVIOR OF ALKALINE LIPOLYTIC ACTIVITY IN HUMAN ADIPOSE TISSUE*

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(Received October 17th, 1966)

SUMMARY

(1) Alkaline lipolytic activity (ALA), a soluble esterase found in human adipose tissue, has been visualized on starch gel after electrophoresis by a staining method which employs naphthol esters as substrates and Fast Blue B as a coupling agent.

(2) The electrophoretic phenotype is manifest as five bands of esterase activity (ALA 1–5) with similar substrate and inhibitor specificity, consistent with the isoenzyme concept.

(3) The effects of time and temperature suggest that ALA 5 is a subunit of ALA 1–4.

(4) 182 fresh adipose tissue preparations have been carefully studied and no genetic variations observed.

(5) The characteristic electrophoretic bands of ALA have also been found in human liver, lung, heart, kidney, adrenal, spleen, thyroid, ovary, testis, brain and muscle.

(6) Their physiological function is not known, but their ability to hydrolyze emulsions of long-chain fatty acid esters suggests a role in lipolysis.

INTRODUCTION

Evidence has previously been presented for the existence in human adipose tissue of at least two lipolytic activities other than lipoprotein lipase[1,2]. These have been named neutral lipolytic activity (NLA) and alkaline lipolytic activity (ALA) according to the pH of the assay system, 7.0 and 8.0 respectively.

The identification of enzymatic proteins after starch-gel electrophoresis in

Abbreviations: ALA, alkaline lipolytic activity; NLA, neutral lipolytic activity.

* Presented in part at the Federation of American Societies for Experimental Biology, April 1965 and April 1966.

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recent years has resulted in the recognition of many genetic variants, the identification of a number of polymorphic systems, and the discovery of isoenzymes. The staining methods employed for the recognition of the enzymatic proteins after electrophoresis may be relatively specific such as those used for lactate dehydrogenase, malate dehydrogenase, glucose-6-phosphate dehydrogenase, *etc.*, or they may be relatively non-specific, as in the case of those used to stain many esterases. Likewise, enzymatic assay systems *in vitro* may be very specific, or measure the enzymatic functions of more than one protein.

The ability to identify enzymatic proteins after starch-gel electrophoresis suggested that a combination of this technique with the quantitative assay for lipolytic activity might elucidate the number and nature of the enzymatic proteins associated with lipolytic activity in human adipose tissue.

In experiments preliminary to the work to be reported here, it was determined that human adipose tissue preparations are capable of hydrolyzing α-naphthyl acetate. Experiments were then designed to determine if this hydrolysis is a manifestation of NLA, ALA, or other enzymes in human adipose tissue, and if this hydrolysis could locate lipolytic activity after starch-gel electrophoresis. The results to be presented in this paper showed that the major portion of the hydrolysis of α-naphthyl acetate is due to ALA which exists as five electrophoretically separate isoenzymes. This suggested further experiments which have provided data on: (1) the frequency with which these isoenzymes occur, (2) the effect of several substrates and inhibitors on the staining of ALA, (3) the effect of time and temperature on the isoenzymes of ALA, (4) the constancy of the electrophoretic phenotype of ALA, (5) the occurrence of these isoenzymes in tissues other than human adipose tissue, and (6) the identity of other esterases found in human adipose tissue.

MATERIALS AND METHODS

Source of tissue

Human adipose tissue was obtained as soon as possible after the induction of general anesthesia for major surgery. Although most of the samples of adipose tissue used in these experiments were obtained from the subcutaneous abdominal wall, in several instances the mesenteric depot was the source. As will be shown subsequently, the electrophoretic properties of ALA on starch block and starch gel were identical for both sources of adipose tissue.

In a separate group of experiments, small samples (10–50 mg) of subcutaneous adipose tissue were obtained from obese children, their siblings and parents by means of an electric drill adapted for skin biopsy[3]. These obese children were under the observation of the Endocrine Clinic at the Buffalo Children's Hospital and were all considered to be free of obvious endocrine disorders.

Various tissues in addition to adipose tissue were also obtained from several individuals 4–20 h *post mortem*.

Preparation of tissue

Approx. 200 adipose tissues and the tissues from several *post mortem* examinations were subjected to starch-gel electrophoresis after preparation as previously described[2]. To eliminate the effects of time and temperature, the following method

was used to prepare 182 additional adipose tissue samples: 300 mg of tissue per ml of 0.15 M KCl were homogenized by hand in a glass tissue grinder for 4 min at 4°. This was centrifuged at 30 000 × *g* for 10 min, stored at 4°, and then applied to the starch gel within a few hours of surgical removal.

Tissue preparations to be exposed to starch-block electrophoresis, eluted, and subsequently analyzed for NLA, ALA and electrophoretic migration on starch gel were prepared in the following manner: 1 g of adipose tissue per ml of 0.15 M NaCl was homogenized in a Waring blendor for 20–30 sec, centrifuged at 800–1000 × *g* for 30 min, and the aqueous middle layer was recovered and used as the source of enzymatic activity.

Starch-block electrophoresis

The method employed for starch-block electrophoresis was a modification of that described by KUNKEL[4]. The experiments to be reported employed two different buffer systems: 0.008 M phosphate–citrate buffer (pH 7.0) (see ref. 5); and 0.1 M barbital buffer (pH 8.6) (see ref. 5).

Starch blocks measuring 76.2 cm × 30.5 cm × 1.3 cm were prepared in a glass mold. A trough 25.4 cm long and 0.16 cm wide was cut across the center of the block.

An adipose tissue preparation, equivalent to 15 g of tissue, was dialyzed for 24 h at 4° against the buffer to be used, after which it was placed in the trough, and the electrophoresis was carried out at 4°.

With 0.008 M phosphate–citrate buffer (pH 7.0) a gradient of 300 V was applied to the block for 20 h, and with 0.1 M barbital buffer a gradient of 250 V was applied for 44 h. To prevent pH changes from occurring on the block, the two outer-end trays were exchanged approximately every 8 h during the electrophoresis.

At the end of the electrophoresis, a 2.54-cm section was removed from each end of the block and discarded. The remaining 71.1 cm were cut into 2.54-cm sections and numbered 1 through 28 from the cathodal end. Each section was eluted in a coarse fritted disc funnel with 8 ml of the phosphate–citrate or barbital buffer used during electrophoresis. The eluates were tested for protein content, lipolytic activity, and migration of lipolytic activity on starch gel by techniques to be described.

Protein determination

Proteins were determined by a Folin method[6] and expressed as μg per ml of eluate.

Assay of alkaline and neutral lipolytic activities (ALA and NLA)

The eluates were analyzed for ALA and NLA by methods previously described[2], and the results expressed as μequiv of free fatty acid[7] released per ml of eluate per h. Basically, the alkaline assay system contained tributyrin at a pH of 8.0 and the neutral assay system contained olive oil at a pH of 7.0. The alkaline system was incubated at 47° and the neutral system at 37° for 30 min.

In one group of experiments, the adipose tissue preparation was replaced with an equivalent amount of starch gel cut from one of several areas after electrophoresis. The gel was then homogenized in the assay system which was analyzed for the release of free fatty acids during incubation.

α-Naphthyl acetate hydrolysis

The hydrolysis of α-naphthyl acetate or other naphthol esters was used in two ways: (1) to locate qualitatively lipolytic activity in the eluates of the starch block, and (2) to identify lipolytic activity on a starch gel after electrophoresis.

In the first instance, one drop of each eluate from the starch block was placed on a strip of Whatman No. 2 chromatographic paper. This was then placed in a solution containing 5 mg of α-naphthyl acetate dissolved in 0.25 ml of acetone, 10 mg of Fast Blue 2B salt, and 25 ml of 0.067 M phosphate buffer (pH 7.5) (see ref. 5). After incubation at 37° for 30 min, the paper strips were washed and observed for the presence of a black precipitate which indicated hydrolysis of α-naphthyl acetate at the spot where enzymatic activity was located. When present, this black precipitate was graded from trace to 4+. In the second instance, the cut surface of a starch gel after electrophoresis was treated in the same manner.

Starch-gel electrophoresis

Vertical starch-gel electrophoresis was performed according to the method of SMITHIES[8,9]. The end trays contained 0.165 M phosphate–citrate buffer (pH 7.0) (see ref. 5). A 1:20 dilution of the same buffer was used in preparing the gel. 0.1-ml samples were placed at the origin in 12 mm × 7 mm × 1 mm slots. The gel was exposed to a voltage gradient of 8 V/cm for 17 h at 4°.

After electrophoresis, the gel was sliced horizontally into two sections. The cut surfaces of the gel were examined for their ability to hydrolyze α-naphthyl acetate in the manner previously described.

In one set of experiments, the conditions of the starch-gel electrophoresis were modified in two ways: (1) The sample slots were cut to contain 0.4 ml (12 mm × 7 mm × 5 mm), and (2) the adipose tissue preparations contained 1 g of tissue per ml of 0.15 M NaCl. After electrophoresis, a longitudinal section was cut from the gel and stained using α-naphthyl acetate. After realignment of the stained with the unstained sections, blocks (12 mm × 7 mm × 5 mm) of the unstained gel were cut corresponding to the five areas diagrammed in Fig. 1. These sections were then assayed for ALA and NLA as described above.

α-Naphthyl propionate, α-naphthyl butyrate, α-naphthyl valerate, α-naphthyl caprylate, α-naphthyl laurate, β-naphthyl acetate, β-naphthyl propionate, β-naphthyl butyrate and β-naphthyl stearate were substituted for α-naphthyl acetate in some experiments. The long-chain fatty acid esters were emulsified in the presence of 40 mg% sodium cholate by rapid injection into a constantly agitated buffer.

A staining technique employing $1.3 \cdot 10^{-3}$ M indoxyl acetate in 0.008 M phosphate–citrate buffer (pH 7.0) was occasionally used[10].

Acetylthiocholine iodide hydrolysis was used in a staining method to identify cholinesterases[11].

When inhibitors such as NaF and eserine sulfate were used in the stains, the gel was first incubated for 20 min in an inhibitor solution without substrate, and then in a reaction mixture to which the inhibitor was added.

RESULTS

After the electrophoresis of human adipose tissue preparations on starch gel and the subsequent staining for esterase activity using α-naphthyl acetate as the sub-

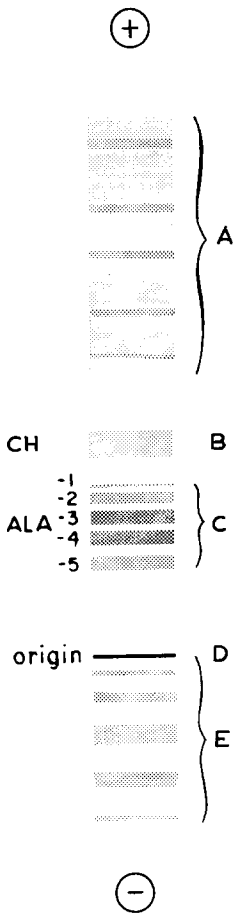

Fig. 1. Esterase zymogram of human adipose tissue. This diagram represents a composite of the esterase zymograms that resulted from the staining of starch gels after the electrophoresis of human adipose tissue preparations. The anode is to the top of the figure. The origin represents the point of insertion of the sample .The capital letters A–E group the bands of enzymatic activity which behaved similarly toward certain substrates and inhibitors.

strate, as many as 17 bands of esterase activity may be seen. These have been diagrammed in Fig. 1. The bands have been grouped into five areas (A–E). Within each area, the bands appear to have almost identical substrate and inhibitor specificities.

The results of the studies on each area will be presented separately beginning with Area C, with which this report is primarily concerned.

Analysis of Area C

Starch-block electrophoresis: When human adipose tissue preparations were subjected to starch-block electrophoresis at pH 7.0, ALA migrated toward the cathode less rapidly than hemoglobin; albumin migrated toward the anode. Fig. 2 shows the results of one experiment in which 38% of the protein and 39% of the original ALA was recovered. Eluates 11–15 were the only ones which contained ALA and, as can be seen, the qualitative α-naphthyl acetate hydrolysis roughly paralleled the ALA re-

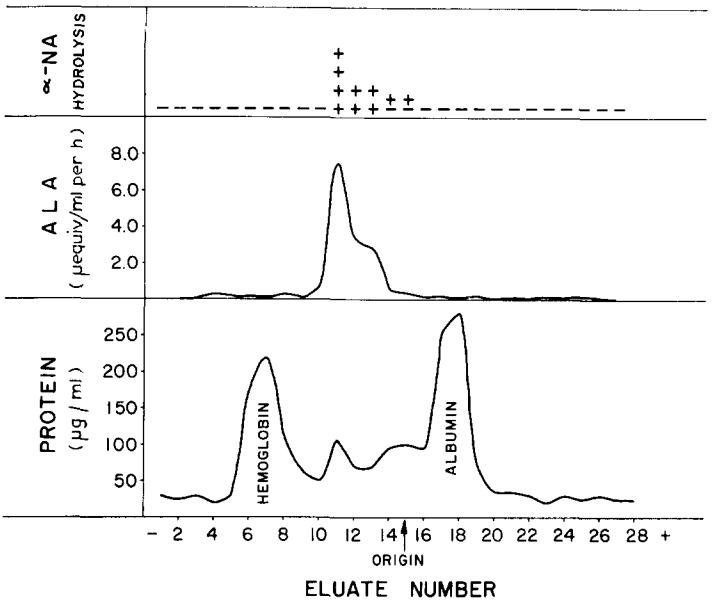

Fig. 2. Starch-block electrophoresis at pH 7.0. A cell-free preparation representing 15 g of human adipose tissue was dialyzed and subjected to starch-block electrophoresis in 0.008 M phosphate-citrate buffer (pH 7.0) as described in the section on methodology. The block was sectioned into 28 strips measuring 2.54 cm in width. These were eluted with 0.008 M phosphate–citrate buffer (pH 7.0) and analyzed for protein content, alkaline lipolytic activity (ALA), their ability to split α-naphthyl acetate (α-NA), and the migration of lipolytic activity on starch gel.

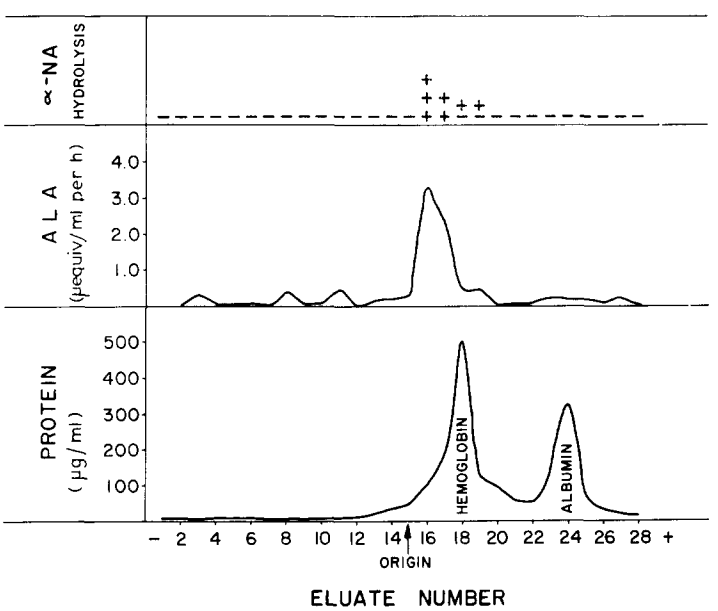

Fig. 3. Starch-block electrophoresis at pH 8.6. A cell-free preparation representing 15 g of human adipose tissue was dialyzed and subjected to starch-block electrophoresis in 0.1 M barbital buffer (pH 8.6) as described in the section on methodology. The block was sectioned into 28 strips measuring 2.54 cm in width. These were eluted with 0.1 M barbital buffer (pH 8.6) and analyzed for protein content, alkaline lipolytic activity (ALA), their ability to split α-naphthyl acetate (α-NA), and the migration of lipolytic activity on starch gel.

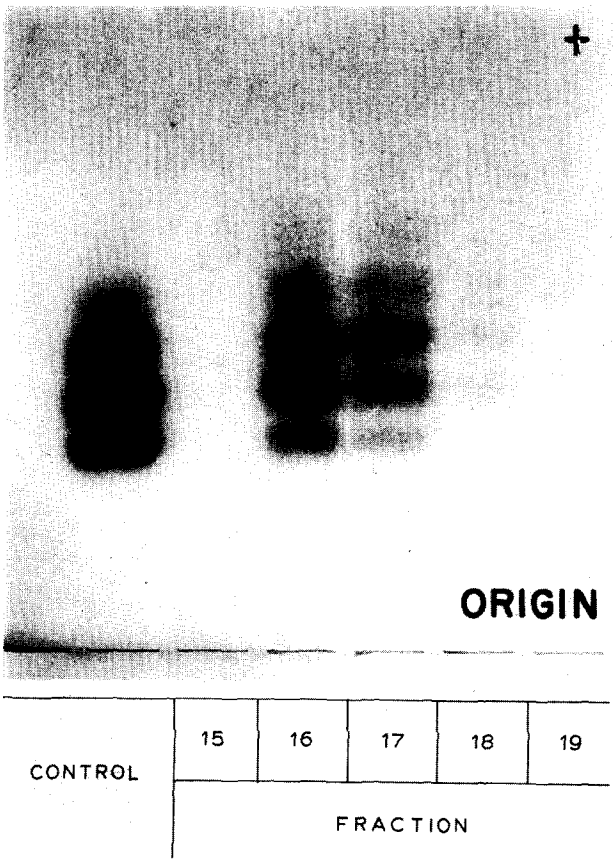

Fig. 4. Starch-gel electrophoresis of eluates from starch block. The material put on each starch block (control) and the eluates obtained after electrophoresis were subsequently subjected to starch-gel electrophoresis as described in the section on methodology. The starch gel pictured above was obtained by using fractions from the starch-block electrophoresis pictured in Fig. 3 (pH 8.6), and is representative of all similar experiments. Lipolytic activity is localized as bands of black precipitate which are the indirect result of the hydrolysis of α-naphthyl acetate.

covered. Although NLA was present in the adipose tissue preparations, none was recovered in any of the eluates of this experiment or any other starch-block electrophoresis.

Fig. 3 shows the results of a starch-block electrophoresis at pH 8.6 in which 23% of the original ALA and 42% of the protein was recovered. ALA migrated toward the anode less rapidly than both hemoglobin and albumin under these conditions. Only eluates 16–19 hydrolyzed α-naphthyl acetate and this paralleled the ALA recovered.

Starch-gel electrophoresis: Fig. 4 shows the stained starch gel after electrophoresis of eluates 15–19 from the above experiment (Fig. 3). It demonstrates that, as with an appropriate control, a series of four or five bands of black precipitate were produced by eluates 16–19 with intensities roughly paralleling the amount of ALA present. The eluates containing ALA from nine starch-block experiments always produced a similar series of bands, which were named ALA 1–5.

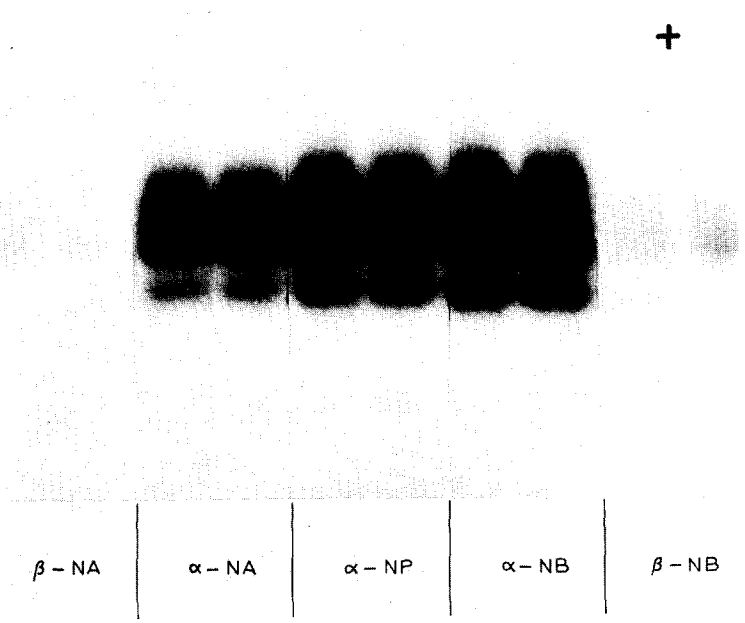

Fig. 5. Comparison of various substrates for identification of lipolytic activity on starch gel. A fresh human adipose tissue preparation was subjected to starch-gel electrophoresis as described. The gel was cut longitudinally into strips, each of which contained two sample slots. The hydrolysis of the following substrates was compared: β-naphthyl acetate (β-NA), α-naphthyl acetate (α-NA), α-naphthyl propionate (α-NP), α-naphthyl butyrate (α-NB), or β-naphthyl butyrate (β-NB).

Recovery of ALA after starch-gel electrophoresis: Analysis for lipolytic activities of the starch gel after electrophoresis proved that Area C contained ALA. The results of one such study in which 61% of the applied ALA was recovered is recorded in Table I. 86% of the ALA recovered in this experiment was found in Area C. Although the amount of NLA recovered was small, 83% of that recovered remained at the origin.

Substrates and inhibitors: Other naphthol esters were compared as substrates for the enzymatic staining of ALA (Fig. 5). For all five bands, α-naphthyl propionate, butyrate and valerate were better substrates than α-naphthyl acetate. β-Naphthyl esters were generally less active substrates than their corresponding α-naphthyl esters. Slow staining occurred when α-naphthyl myristate, α-naphthyl caprylate, α-naphthyl laurate and β-naphthyl stearate were used as substrates. When indoxyl acetate was used as the substrate, the ALA bands stained slowly with the insoluble reaction product (indigo) formed.

Since it is known that ALA is 90% inhibited by 10^{-2} M NaF (ref. 2) and only 10% by 10^{-4} M eserine*, the effect of these chemicals on the staining of ALA was studied. Fig. 6 demonstrates the marked reduction in staining of ALA in the presence of 10^{-2} M NaF and the lack of inhibition of 10^{-4} M eserine. Acetazolamide (10^{-5} M), EDTA (10^{-3} M), and $CaCl_2$ (10^{-5} M) did not inhibit the staining of the 5 ALA bands.

Effect of time and temperature: ALA is a relatively stable enzyme. When human

* J. D. SCHNATZ AND J. A. CORTNER, unpublished data.

TABLE I

ANALYSIS OF STARCH GEL FOR ALKALINE LIPOLYTIC ACTIVITY AFTER ELECTROPHORESIS

Sections (12 mm × 7 mm × 5 mm) of starch gel were cut from the Areas A–E (depicted in Fig. 1) and assayed for ALA. In this experiment, 61% of the applied ALA was recovered.

Area	*% ALA*
A	0.5
B	1.7
C	86.0
D	9.1
E	1.8

adipose tissue preparations were stored at temperatures varying from −65° to +25° for one week, only small losses of ALA were recorded. After standing at room temperature for one week, the first effect seen was an increase in Band 5 with no loss of activity. Subsequently, slower bands developed but retained stainable esterase activity and the majority of the measurable ALA. Although not objectively studied, increases in Band 5 were noted to occur with repeated freezing and thawing of specimens, and on occasion even one freeze and thaw would produce some increase in Band 5 as compared to the specimen kept at 4° for the same period of time. The upper portion of Fig. 7 shows an experiment where no loss of activity was recorded. The

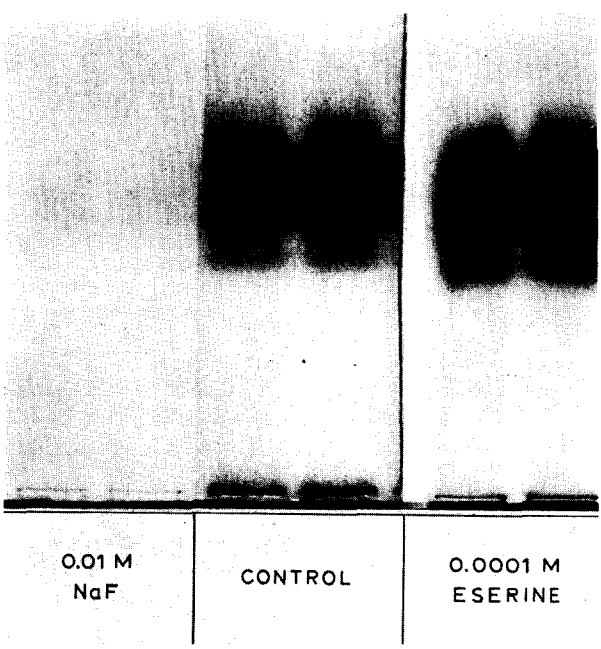

Fig. 6. Effect of NaF and eserine sulfate on stainable lipolytic activity. Adipose tissue was prepared and subjected to starch-gel electrophoresis as described in the text. After electrophoresis, the starch gel was incubated for 20 min at 37° in 0.067 M buffer (pH 7.5), which contained 0.01 M NaF or 0.0001 M eserine sulfate. Finally, the gels were placed in a solution of α-naphthyl propionate and Fast Blue B to which similar concentrations of NaF or eserine sulfate were added. The results are compared above with a starch gel stained in the normal fashion.

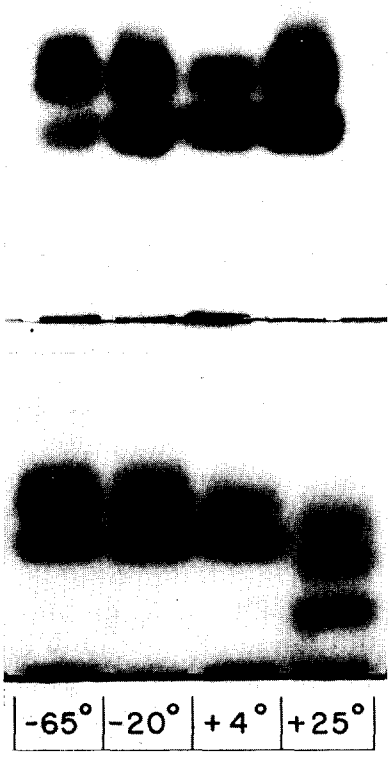

Fig. 7. Effect of time and temperature on the electrophoretic mobility of lipolytic activity. Adipose tissue preparations were divided into aliquots which were stored for one week at −65°, −20°, 4° and 25° prior to electrophoresis on starch gel. In the experiment depicted in the upper half, no loss of activity was recorded after one week. The experiment demonstrated in the lower half shows the development of slower migrating bands in another specimen that had remained at room temperature for one week at which time 83% of the measurable ALA was still present.

lower half of Fig. 7 demonstrates the development of slower bands of enzymatic activity which herald the degradation of ALA. In this experiment, the slower bands developed after one week at room temperature. At that time, 83% of the measurable ALA was still present and no loss had occurred at the lower temperatures.

Clinical studies: 182 carefully controlled human adipose tissue preparations were studied by starch-gel electrophoresis as described in the section on methodology. No variation in the migratory rate of the 5 bands of ALA was noted. Only Bands 3 and 4 were always present as noted in Fig. 8. Band 5 was present in 50% of the specimens and Bands 1 and 2 in 23% and 80% respectively. As described in METHODS, approx. 200 additional specimens of human adipose tissue have been studied with less rigid control, and only one variant in the migratory pattern was observed. Three slower bands and no normal bands were present. Subsequently, a skin biopsy was obtained from this patient and a repeat electrophoresis performed on the attached adipose tissue. This time, the ALA pattern was perfectly normal.

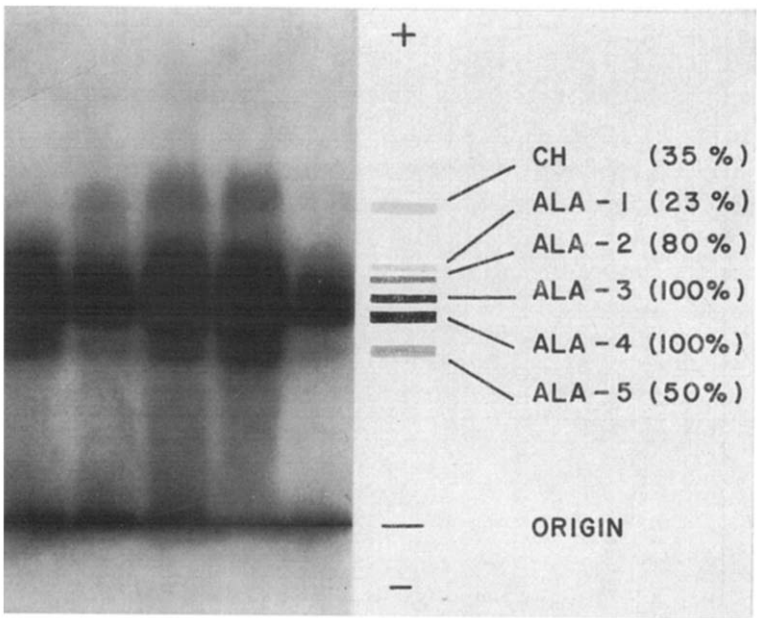

Fig. 8. Representative patterns seen on starch gel after electrophoresis of human adipose tissue. Fresh human adipose tissues obtained at surgery from 182 individuals were homogenized in 0.15 M KCl (300 mg/ml) and centrifuged at 30 000 × *g* for 10 min. The aqueous middle layer was subjected to starch-gel electrophoresis and the lipolytic activity identified as described in the text. Pictured above is an area of a starch gel which extends from the origin to approx. 8 cm toward the anode. This area consistently has certain characteristic bands which for reasons detailed in the text have been designated as Ch and ALA 1–5. A schematic representation of these bands is seen to the right. The frequency with which these bands occurred in 182 surgical specimens is noted in the parentheses to the far right of the figure.

Although most specimens were obtained soon after induction of anesthesia, no effect from prolonged anesthesia and no variations due to the type of anesthesia were noted.

In addition to the surgical specimens examined, the adipose tissues of 10 obese children and 11 of their family members of normal weight were examined by performing a punch skin biopsy using an electric drill[3]. Typical electrophoretic mobilities were noted in all cases. Quantitative assays were not performed because of the small size of the specimens obtained.

Two lipomas from different people were found to have ALA patterns and assays identical with their subcutaneous fat. In three different people, mesenteric and subcutaneous adipose tissues were examined and found to have identical starch-gel patterns of ALA.

Other tissues: When human tissues obtained at *post-mortem* examination were subjected to starch-gel electrophoresis, esterase bands with migratory rates identical to ALA were found in liver, lung, heart, kidney, adrenal, spleen, thyroid, ovary, testis, brain and muscle. Attempts were made to correlate the assayable ALA with the intensity of the stained ALA bands. Poor correlation was observed for some tissues. The ALA bands were not detected in erythrocytes or leukocytes. When α-naphthyl butyrate or α-naphthyl valerate were used as substrates, faint ALA bands were usual-

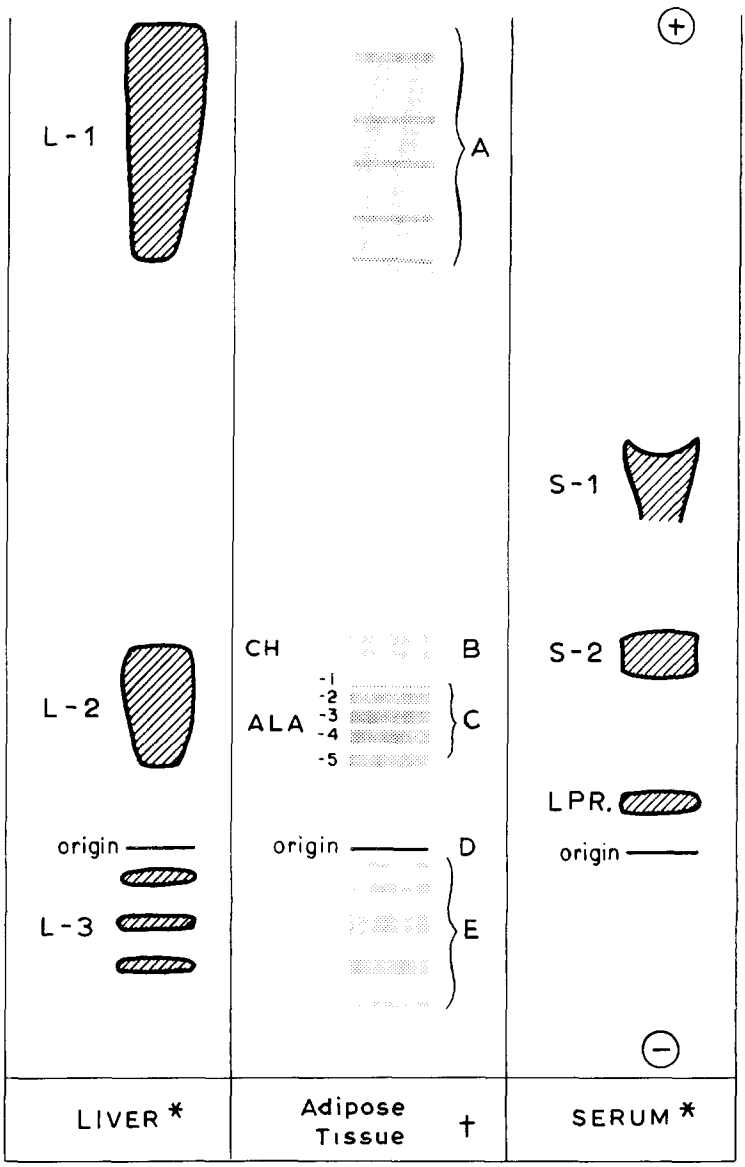

Fig. 9. Esterase zymogram of human adipose tissue compared with that of liver and serum. Adipose tissue was prepared as described and subjected to starch-gel electrophoresis and staining. As many as 17 bands of esterase activity were observed and grouped into 5 major areas (A–E). The esterase zymogram of human liver and serum obtained by ECOBICHON AND KALOW[17] are depicted for comparison. *, results of ECOBICHON AND KALOW[17]; †, present work.

ly seen in serum. Concentrated urine (500 fold) did not contain ALA. Homogenates of pancreas and a preparation of lyophilized human pancreatic lipase* contained esterase activity which migrated differently from ALA.

* Kindly supplied by Dr. EDMUND KLEIN.

Analysis of Area B

A single band of activity (Ch) was observed in Area B (Figs. 1 and 8) in 35% of the human adipose tissue preparations examined. It had a migratory rate equal to that of serum pseudocholinesterase at several pH values and was inhibited by 10^{-4} M eserine sulfate. The protein responsible for this esterase activity also hydrolyzed acetylthiocholine iodide in a stain specific for cholinesterase. For these reasons, it was labeled Ch (Figs. 1, 8, 9).

Analysis of Area A

Area A (Fig. 1) consisted of a large area of diffuse staining which overlapped albumin and prealbumin. Within this area as many as 5 bands of more intense staining were observed when using α-naphthyl acetate as the substrate after these electrophoretic conditions. An increased rate of staining was noted with α-naphthyl propionate, α-naphthyl butyrate and β-naphthyl acetate. 10^{-4} M eserine sulfate caused moderate inhibition of the staining of Area A. In comparison with the previously stated frequency of occurrence of ALA bands 1–5 in Area C, Area A stained in 47% of tissues studied at a concentration of 300 mg/ml. However, all adipose tissue examined at a concentration of 1 g/ml produced staining in Area A. In addition to their presence in human adipose tissue, these esterases were found in homogenates of human heart, liver, kidney, muscle, thyroid and adrenal. They were not found in lung, brain, testis, ovary or pancreas.

Analysis of Area E

Only 40% of the human adipose tissues examined in a concentration of 300 mg/ml produced staining in Area E (Fig. 1) when α-naphthyl acetate was used as the substrate. However, all specimens studied in a concentration of 1 g/ml produced staining in this area. No substrate other than α-naphthyl acetate produced significant staining in this area. These esterases were found in human heart, liver, kidney, spleen, lung, gonads, thyroid, adrenal, lymph nodes and brain. They were not found in muscle or pancreas. These esterase bands were the only ones noted in two normal urines which had been concentrated 500 fold.

DISCUSSION

Human adipose tissue preparations were subjected to vertical starch-gel electrophoresis and subsequently stained for esterase activity by a histochemical staining technique which was first used in histopathology[12–14], adapted for starch-gel work by HUNTER AND MARKERT[15], and subsequently used in demonstrating human tissue esterases by many investigators[16–23]. Many different α- and β-naphthol esters can be used, and esterases can be partially classified by the results obtained. With this technique, as many as 17 dark precipitate bands could be seen in cell-free preparations of human adipose tissue. These bands have been grouped into 5 areas, each of which represents an enzyme or a group of enzymes of similar substrate and inhibitory specificity compatible with the isoenzyme concept. The data presented here are primarily concerned with Area C which has been shown to be associated with ALA.

The first evidence for the relationship between Area C and ALA was obtained from starch-block electrophoresis experiments (Figs. 2 and 3), in which the occurrence

of measurable ALA in certain fractions correlated with the ability to hydrolyze α-naphthyl acetate. This ability to hydrolyze α-naphthyl acetate was seen in as many as 5 precipitate bands of activity in Area C (Fig. 4). These bands were similar to those seen after starch-gel electrophoresis of a typical adipose tissue preparation (Fig. 8).

Direct analysis of the starch from Area C then proved that this area actually contained ALA (Table I).

182 fresh surgical specimens of human adipose tissue were then studied on starch gel using identical concentrations of tissue. ALA bands 3 and 4 were always found, Band 5 occurred in 50% of the specimens, and Bands 1 and 2 were present in 23% and 80%, respectively. Bands 3 and 4 were also the most intensely staining bands, suggesting that the frequency distribution could be due to variations in the total activity. Although this distribution is compatible with two subunits freely associating into tetramers, as is the case with lactate dehydrogenase[24], the elution of ALA from Sephadex G-200 in two major peaks[25] is compatible with a dimer–monomer relationship. The fact that Band 5 predominates in the second peak in contrast with the predominance of Bands 1–4 in the first peak suggests that Band 5 is a subunit which retains activity. The degradation studies presented in this paper are also compatible with this concept.

To summarize the present status regarding human adipose tissue ALA, it can be said that ALA is one of several lipolytic activities present in human adipose tissue, and that certain features have led to the suggestion that it is an esterase[2]. The present studies not only demonstrate that ALA can be detected after electrophoresis on starch gel, but also indicate that it is a soluble aliesterase with increasing affinity for α-naphthyl propionate, α-naphthyl butyrate and α-naphthyl valerate. The ability to hydrolyze an emulsion of β-naphthyl stearate may indicate a physiological role in the hydrolysis of long-chain fatty acid esters.

As measured in cell-free preparations of adipose tissue, the assay for ALA is relatively specific. Most of the ALA can be separated from NLA (ref. 25), and direct analysis of a starch gel after electrophoresis (Table I) suggests that Areas A, B and E contribute little to cross-reacting assay results. Of interest is the fact that some ALA is recovered at the origin (Area D) of a starch gel after electrophoresis (Table I). Some ALA is also present in the only Sephadex fractions which result in staining at the origin[25]. Two possible explanations for this failure to migrate in the electrophoretic field are that some ALA exists as a large molecule such as a lipoprotein complex or a polymer.

The isoenzymes of ALA have been shown to be present in other tissues (see RESULTS) by demonstration of the characteristic Bands 1–5. The poor correlation between these bands and the measurable ALA of tissues other than adipose tissue suggests that the ALA system measures some esterases in addition to ALA 1–5. Thus, although relatively specific for the study of adipose tissue, the ALA system appears to be less specific when analyzing other tissues.

The characteristic bands of enzymatic activity which have been demonstrated in this paper to be associated with ALA of human adipose tissue have not previously been shown to be present in this tissue. The fact that they were also present in other tissues is verified by analysis of electrophoretic patterns obtained by other investigators[15–21]. Fig. 9 shows a comparison of the 17 bands seen in human adipose tissue with a diagram of the bands seen by ECOBICHON AND KALOW[17] in human liver and serum.

Their electrophoretic method was similar to that used in these studies, allowing this comparison. As can be seen, the area designated L-2 by ECOBICHON overlaps Area C (ALA 1–5) and Area B. Evidence has been obtained which suggests that Area B is pseudocholinesterase, present in approximately one-third of the tissues examined, presumably by virtue of the serum present in the adipose tissue preparations. Its migratory rate compares with that of S-2 (Fig. 9), known to be serum pseudocholinesterase.

Area A corresponds to what ECOBICHON refers to as L-1 of liver. This group of esterases all have the characteristics of aliesterases and have been observed in numerous tissues by several authors[15–20]. Area E corresponds to the acetylesterases which ECOBICHON refers to as L-3. They, too, are probably isoenzymes present in many tissues and have also been observed by others[15–20].

The characterization of L-1 as an aliesterase[17] is consistent with the substrate studies carried out on Area A of human adipose tissue. Similarly, the classification of L-3 as a group of acetylesterases is consistent with the fact that only α-naphthyl acetate acted as substrate for Area E of human adipose tissue.

None of the above described esterases has a known function. ECOBICHON did observe, however, that the enzymes in Area L-2 were activated by sodium cholate toward the hydrolysis of long-chain fatty acid esters, indicating a potential lipase type of activity. This finding is consistent with the present substrate studies.

BLANCO AND ZINKHAM[21], in reporting on human tissue esterases, observed a variant of the liver esterases located in the area which they call central zone. Unfortunately, they used *post-mortem* tissue and were not able to retest the individual or the family.

Because of the possibility that the "central zone" described by BLANCO AND ZINKHAM was the same as Area C (ALA 1–5) presently under discussion, additional studies were performed. Correction for the difference in methods proved them to be the same group of esterases. The variant demonstrated by them could conceivably be due to the nature of the sample since, as noted under RESULTS, bands of slower migration appear as tissue is allowed to stand. Furthermore, the present studies provide data on one individual who was found to have a slower migratory pattern when tissue obtained at surgery was examined. Although examined within 24 h of surgery, degradation had apparently occurred since a repeat examination of a fresh specimen revealed a normal pattern. This finding stimulated the carefully controlled study of 182 freshly prepared surgical specimens which revealed no variants.

The absence of a demonstrated phenotypic variation in ALA bands 1–5 includes analysis of 11 markedly obese children and two benign lipomas, in addition to the 182 carefully controlled surgical specimens.

The isoenzymes of ALA are present in a number of human tissues including adipose tissue. Their physiological function is presently unknown. The discovery of a genetic variant associated with altered physiology or disease could lead to knowledge of their physiological role.

ACKNOWLEDGEMENTS

We wish to thank Drs. ALEXANDER BEARN and BARTON CHILDS for reviewing the manuscript, Dr. THOMAS ACETO for permission to obtain the punch biopsy

specimens from the patients of the Endocrine Clinic at the Buffalo Children's Hospital, and Mr. JOHN M. BUCHHEIT, Mrs. GERTRUDE SCHANKER, Mrs. ARLENE FORMAN and Mr. ROBERT G. SMITH for technical assistance. The continued interest and support of our many surgical colleagues is gratefully acknowledged.

This work was supported in part by Grant G-64-RP-6 from the United Health Foundation of Western New York, Grants AM-06872 and AM-05581 from the National Institute of Arthritis and Metabolic Diseases, and by funds contributed to Health Research, Inc., by the American Legion Auxiliary, Department of New York, Inc.

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