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A Comparative Study of Alkaline Lipolytic Activity in Adipose Tissue of Various Mammals* (33528)

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Human adipose tissue contains a soluble esterase called alkaline lipolytic activity (ALA) (1, 2). This activity can be quantitated by measuring the free fatty acid (FFA) released from tributyrin (pH 8.0) at 47° and qualitatively identified after starch gel electrophoresis by using naphthol esters as substrate and fast blue 2B salt as coupling agent. The latter technique has demonstrated that human adipose tissue ALA exists as five electrophoretically separate isozymes (3). The present paper reports the use of the same techniques to analyze adipose tissue of other mammals.

Methods. Tissue was obtained from several fat depots[1] of nine mammals within 2–3 hr after sacrifice (Table I). The tissue was frozen at −65°[2] until ready for comparison with extracts of human tissue stored in the same manner.

For study, the tissue was thawed, homogenized in cold 0.15 *M* KCl in a glass tissue grinder and centrifuged at 25,000*g* for 10 min after which the aqueous middle layer was recovered and used as the source of enzymatic activity. In order to obtain similar staining after starch gel electrophoresis, the concentration of adipose tissue was varied from 100–1,500 mg per ml of KCl.

Vertical starch gel electrophoresis of the adipose tissue extracts was performed according to the method of Smithies (4). The end trays contained 0.165 *M* phosphate–citrate buffer (pH 7.0) and a 1:20 dilution of the same buffer was used in preparing the

TABLE I. Alkaline Lipolytic Activity (ALA) in Adipose Tissue Extracts.[a]

Mammal	ALA (μeq of FFA/g/hr)
Cat (O)	171.7
Pig (P)	78.1
Rabbit (S)	43.7
Lamb (S)	37.4
Rat (S)	33.9
Human (S)	30.9
Mouse (S)	28.8
Monkey (O)	6.0
Dog (O)	3.0
Steer (S)	0.4

[a] Adipose tissue extracts were prepared as described in the text and activity quantitated by the assay system previously described for measuring ALA in extracts of human adipose tissue. The rise in FFA during incubation was determined and activity expressed as μeq of FFA/g of tissue/hr. Abbreviations for source of tissue: (O) = omental, (P) = pericardial, (S) = subcutaneous.

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[1] Previous studies on human and rabbit adipose tissue indicated that the enzyme pattern on starch gel was the same for various sources of fat.

[2] Although aqueous extracts undergo minor changes in isozyme pattern, even at −65° [Ref. (3)], unextracted tissue can be frozen at −65° without alteration of the isozyme pattern (unpublished data).

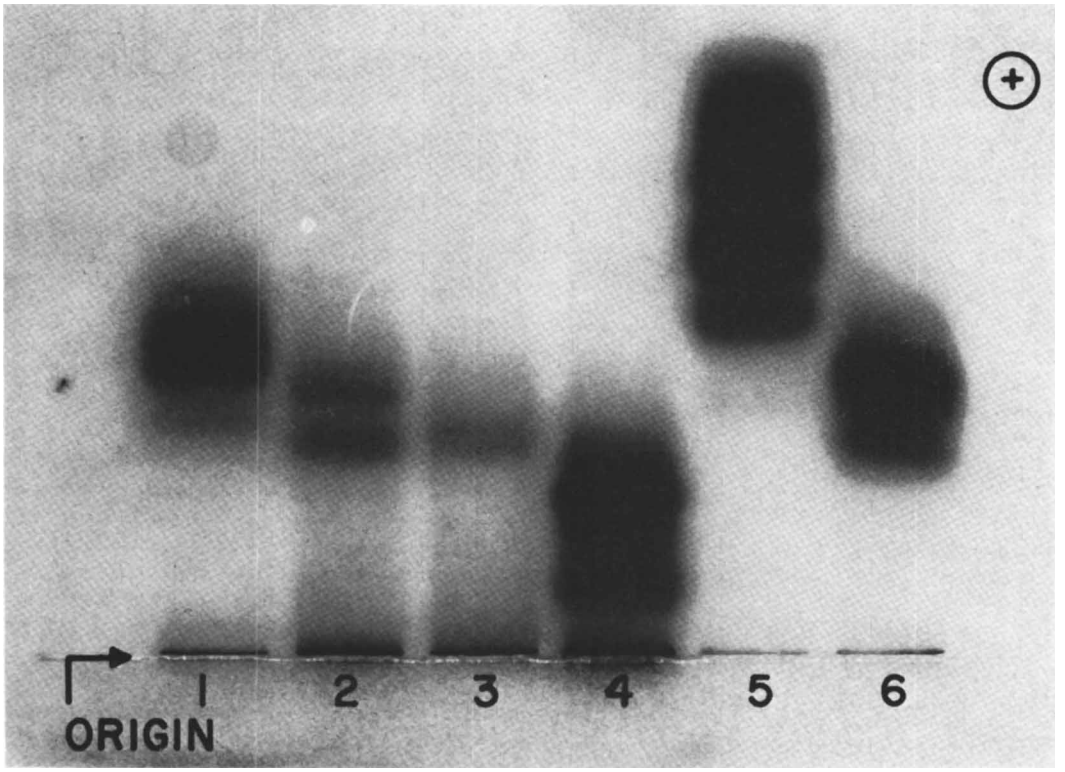

FIG. 1. ALA zymograms of adipose tissue extracts from (1) human, (2) dog, (3) monkey, (4) mouse, (5) pig, (6) rabbit.

gel. Samples (0.1 ml) were placed at the origin in 12 × 7 × 1 mm slots after which the gel was exposed to a voltage gradient of 5 V/cm for 16 hr.

After electrophoresis, the gel was sliced horizontally into two sections. The cut surfaces of the gel were stained for esterase activity using naphthol esters as substrate and fast blue 2B as the coupling agent (3). Although α-naphthyl acetate and α-naphthyl propionate were also used as substrates in the staining reaction, α-naphthyl butyrate gave the best results which are reported here.

Inhibitors such as NaF and eserine sulfate were added to the staining solutions in the concentrations indicated in order to characterize the esterase bands.

Adipose tissue extracts were assayed for ALA in a system which contained tributyrin as substrate at pH 8.0, 47° (2). A rise in free fatty acids (FFA) during incubation was determined by titration (5) and activity was expressed as microequivalents of FFA per gram of tissue per hour.

Results. Of nine mammals whose adipose tissue extracts were subjected to electrophoresis in starch gel and then examined for esterase activity, all but that obtained from the steer contained detectable bands of activity. While the zymograms of all of the other mammals were found to differ from each other and that of man (Figs. 1 and 2), the electrophoretic patterns of human and rabbit adipose tissue were the most similar. The visible bands of esterase activity had characteristics similar to those of ALA in human adipose tissue by virtue of more substrate affinity for α-naphthyl butyrate than for α-naphthyl acetate or propionate, and marked inhibition in 10^{-2} *M* NaF but only mild inhibition in 10^{-4} *M* eserine (3). Adipose tissue of the dog appeared, by staining on starch gel, to have less ALA than the other mammals tested exept for the steer.

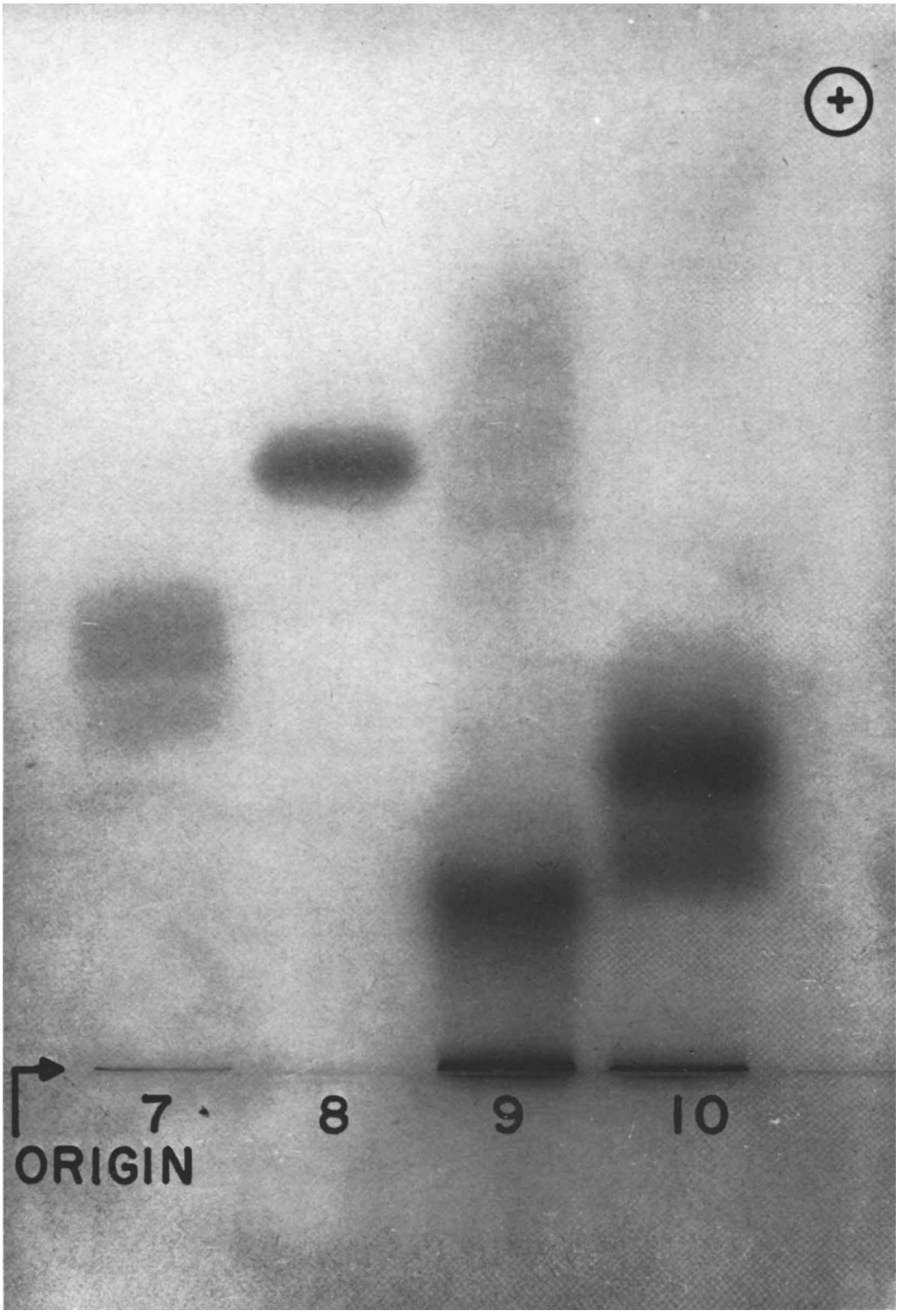

FIG. 2 ALA zymograms of adipose tissue extracts from (7) human, (8) lamb, (9) cat, (10) rat

Enzymatic assay confirmed the low ALA present in adipose tissue extracts of dog and steer (Table I). Four mammals—rabbit, lamb, rat and mouse—had activity which was in a range similar to that of man.

Discussion. Human adipose tissue ALA exists as five isozymes, ALA 1–5 (3), and in three fractions separable by Sephadex gel filtration (6). The esterase activities of the mammalian species reported here were for the most part manifested as several separate bands after starch gel electrophoresis, suggesting that they, too, exist as isozymes.

The different electrophoretic patterns observed suggest alterations in amino acid sequence sufficient to produce a different mobility without altering enzymatic activity appreciably. This is analagous to the species differences in electrophoretic patterns of hormones, lipoproteins, and other enzymes without comparable alteration in physiological function.

No genetic variants of the ALA isozyme pattern were found in over 700 extracts of human adipose tissue examined. Adipose tissue extracts of the rabbit have an electrophoretic pattern (Fig. 1) and Sephadex gel filtration pattern similar to that of extracts from humans (7, 8). A genetic variant of this pattern was demonstrated in the rabbit and is currently under study from both a genetic and metabolic viewpoint.

Summary. The alkaline lipolytic activity (ALA) of human adipose tissue has been characterized previously. The present study reports the variations in quantitative activity and starch gel zymograms in adipose tissue of nine other mammals.

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