by insulin in adipose tissue[36]. The precise nature of the alteration in the surface charge and the mechanism by which it is brought about are not yet known but are currently under investigation.

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Interaction between fluorescent-labeled $ACTH_{1-24}$, isolated fat cells, and serum albumin[1]

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Summary. Part or all of the difference in the ability of $ACTH_{1-24}$ and a fluorescent-labeled $ACTH_{1-24}$ to activate lipolysis in fat cells can be accounted for by label-related binding to albumin present in the assay medium.

Considerable interest has been expressed in the development of molecular probes for use in evaluating hormone/cell-receptor interactions. One such potential probe is DNS-$ACTH_{1-24}$[5], a derivative of adrenocorticotropin-(1-24)-tetracosapeptide in which the sole modification is the introduction of the fluorescent DNS group at the ε-nitrogen atom of lysine-21. According to the available data[6,7], the conformation of DNS-$ACTH_{1-24}$ is the same as that of its parent hormone $ACTH_{1-24}$, i.e., a random coil. DNS-$ACTH_{1-24}$ stimulates activity in adrenal cells and in isolated fat cells to the same extent as the unsubstituted hormone[6,8]. However, the concentrations required to stimulate lipolysis in the fat cell system were considerably higher for the modified hormone in comparison to unlabeled $ACTH_{1-24}$[8]. Here we investigate the possibility that serum albumin, which is necessarily included in the fat cell assay medium as a physiological means of binding the fatty acids released during lipolysis[9], could also bind the labeled hormone and thus reduce its effective concentration in the medium. DNS-Lys was used as a control to estimate the binding due to the DNS group alone.

Materials and methods. DNS-$ACTH_{1-24}$ was a gift from Dr Peter Schiller, and $ACTH_{1-24}$ (Synactin®) was obtained through the courtesy of Dr W. Rittel, CIBA-Geigy Ltd, Basel (Switzerland). DNS-Lys lot 517 was purchased from Fox Chemical Co., and fraction V fatty acid-free bovine serum albumin lot 14 was obtained from Miles Laboratories. Human serum albumin fraction V was from the Swiss Red Cross, Bern (Switzerland), and defatted according to Chen[10]. Both albumins gave similar results. Titrations were made by changing the concentration of albumin in the presence of a fixed quantity of DNS-$ACTH_{1-24}$ or DNS-Lys. Fluorescence intensity and anisotropy measurements were carried out under N_2 on a Weber-type double beam instrument which simultaneously measures the parallel and perpendicular components of the emission and returns the computed values of the anisotropy and the total intensity[11]. The results were corrected for light scattering due to albumin in duplicate experiments with solutions which did not contain fluorescent ligand. Concentrations were determined from absorbance measurements using molar decadic extinction coefficients of 43,600 cm^2/mmole for (bovine)

albumin at 280 nm, and 4600 cm^2/mmole at 330 nm for DNS-Lys (determined in the course of these experiments and identical with the extinction coefficient of DNS-$ACTH_{1-24}$ reported by Schiller[7]).

Results and discussion. Both $ACTH_{1-24}$ and DNS-$ACTH_{1-24}$ activated lipolysis to the same maximal degree. Thus DNS-$ACTH_{1-24}$ presumably activates the same receptors as $ACTH_{1-24}$. However, there was a 20–25-fold difference in the apparent affinities of the native and labeled hormones (table), which could be due to binding of the labeled hormone to albumin.

When albumin was added to dilute solutions of either DNS-$ACTH_{1-24}$ or DNS-Lys, both the emission anisotropy and the total fluorescence intensity increased. The anisotropy leveled off as the albumin concentration was increased, but the total intensity continued to rise. The origin of this apparent discrepancy is inherent in the anisotropy parameter itself, which is a non-linear function of the fraction of ligand in the bound form when the quantum yields of the free and bound species are not identical[12]. The maximum anisotropy was higher for equilibrium-bound DNS-$ACTH_{1-24}$ and DNS-Lys (0.18–0.19) than that estimated for DNS covalently bound to albumin under similar conditions (0.14; calculated from the Perrin equation on the basis of the limiting anisotropy, excited state lifetime, and rotational relaxation time[13]). Therefore the DNS group can be assumed to be rigidly bound to the albumin in both the DNS-$ACTH_{1-24}$ and DNS-Lys complexes.

The saturation fraction s of the fluorescent ligand is defined as the ratio of the bound ligand concentration to the total ligand concentration, and is given by either of the 2 functions[12]

$$s=(I-I_0)/(I_1-I_0) \quad (1)$$

$$s=(\Delta I-\Delta I_0)/(\Delta I_1-\Delta I_0) \quad (2)$$

Here I is the total intensity ($I_\parallel+2I_\perp$) and ΔI is the difference $I_\parallel-I_\perp$ for the mixture of free and bound ligand. The subscripts 0 and 1 refer to the intensities when no protein is present and when sufficient protein has been added to ensure that all the ligand is bound. Both (1) and (2) were used in the analysis of the present data, and essentially identical numerical results were obtained. Since saturation was not reached, it was necessary to estimate the anisotropy and intensity parameters which would have been obtained when all the ligand was bound. This was accomplished by linear extrapolation, at high protein to ligand ratios, of plots of $(\Delta I-\Delta I_0)/[P]_0$ vs $(\Delta I-\Delta I_0)$ and $(I-I_0)/[P]_0$ vs $(I-I_0)$. Under these conditions the concentration of complex is negligible in comparison to the total protein concentration $[P]_0$.

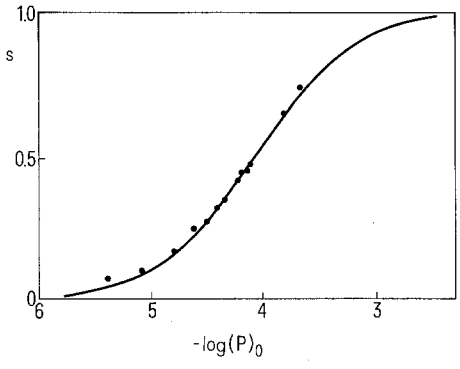
The titration curve for DNS-$ACTH_{1-24}$ is shown in the figure. Using the apparent association constant $k \simeq 12{,}000$ l/mole, the solid line in the figure was calculated from the equilibrium relationship for 1:1 binding

$$s=k[P]/(1+k[P]) \quad (3)$$

where [P] is the concentration of free albumin. The correspondence between the observed and calculated data indicates that a 1:1 binding model appears to be adequate as a first approximation to the titration behaviour.

However, the reader will have noticed that the present binding experiment is a titration of ligand with protein, as opposed to the usual titration of protein with ligand. This is a drawback (imposed in this case by the limited availability of the labeled hormone), and has 2 important consequences. 1st, the experiment may not reflect binding at protein sites which would be saturated at significantly higher total ligand concentrations, and 2nd, no information is available about the number of any such sites. In fact albumin has several sites which are capable of binding DNS or DNS-like chromophores[14,15]. Assuming that binding of DNS-derivatives to cellular components can be neglected and that there are no other competing processes, we estimate on the basis of a single albumin binding site that a maximum of 22% the total DNS-$ACTH_{1-24}$ added in the present fat cell experiments was available for binding to the receptor in the presence of 20 mg of albumin per ml. This implies at least a 4-fold decrease in the concentration necessary to elicit half-maximal activity, and thus it appears that binding of the DNS moiety to even a single albumin site can account for a significant fraction of the difference in concentrations required to reach half-maximal activity. If there are as many as 4 such sites, the available DNS-$ACTH_{1-24}$ would be reduced to 6% of the total added and almost all of the difference in activity would be accounted for. These results suggest that albumin-bound DNS-$ACTH_{1-24}$ does not stimulate cellular activity.

The addition of unlabelled $ACTH_{1-24}$ to mixtures of albumin and DNS-$ACTH_{-24}$ failed to elicit changes in either the intensity or the anisotropy. Therefore the unlabelled hormone does not interact with albumin at the site or sites occupied by the DNS moiety. DNS-Lys was found to bind nearly as well as DNS-$ACTH_{1-24}$, and thus it appears that the DNS group itself is the major contributor in the labeled hormone/albumin interaction. In an assay system containing an extrinsic binding component such as serum albumin, the available or effective concentration of a hormone labeled with a ligand for that component could be significantly lower than the total added.



Fraction of DNS-$ACTH_{1-24}$ bound (s) as a function of the negative logarithm of the added albumin concentration. 4.5 µM DNS-$ACTH_{1-24}$ in 0.1 M phosphate buffer, pH 7.1, under N_2 at 25 °C.

Concentrations necessary for half-maximal release of fatty acids and glycerol from isolated fat cells induced by $ACTH_{1-24}$ and DNS-$ACTH_{1-24}$ in the presence of 2% serum albumin*

Substance released	Concentration at half-maximal activity (moles/l)		Ratio ($ACTH_{1-24}$/ DNS-$ACTH_{1-24}$)
	$ACTH_{1-24}$	DNS-$ACTH_{1-24}$	
Glycerol	3×10^{-9}	6.8×10^{-8}	23
Fatty acids	2×10^{-9}	5.2×10^{-8}	26

* Fat cells isolated by collagenase-treatment[16] from eididymal adipose tissue of Sprague-Dawley rats (100 ± 20 g) were suspended in 2 ml Krebs-Ringer bicarbonate buffer, pH 7.4, with 5 mM glucose and 20 mg/ml defatted human serum albumin (fraction V, Swiss Red Cross) at 37 °C for 40 min. Incubations were stopped by cooling to 0 °C, glycerol was determined enzymaticaly[17] and free fatty acids by titration[18].

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5 Abbreviations used: DNS-$ACTH_{1-24}$, $N^{\epsilon 21}$-(dimethylaminonaphthalene-5-sulfonyl)-adrenocorticotropin-(1-24); DNS-Lys, N^{ϵ}-(dimethylaminonaphthalene-5-sulfonyl-L-lysine; $ACTH_{1-24}$, adrenocorticotropin-(1-24) tetracosapeptide (Synactin®); ANS, 8-anilino-l-naphthalene sulfonate.

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Rapid temperature programmed gas-liquid chromatography of volatile fatty acids (C_1-C_7) for the identification of anaerobic bacteria

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Summary. A gas liquid chromatography method for the separation of 10 volatile fatty acids (C_1-C_7 and isomers) has been improved by using oven temperature programmed conditions. In our conditions, the proprietary stationary phase SP 1220 introduced by Supelco Inc., gave sharp separation of volatile fatty acids in less than 8 min. This method was suitable for analyses with both thermal conductivity and flame ionization detectors.

The first application of GLC for the rapid identification of bacteria was reported in 1963[1]. Gas chromatography is now currently used for the identification of bacterial by-products in broth cultures. Whole culture and culture supernatant[2], products obtained by cation exchange column[3] or by ether extraction[4] and vapor phase of anaerobic cultures[5] can be chromatographed. Volatile fatty acids (VFA), non volatile fatty acids and alcohols produced by anaerobic bacteria may be necessary for their identification and generally the VFA analysis is the first step. Among the different GLC methods used for separation of VFA usually found in anaerobic bacterial cultures, the use of the stationary phase SP 1220 prepared by Supelco Inc. (Supelco, Inc. 1975. Chromatography/lipids. Analysis of VFA's from anaerobic fermentation. Bulletin 748E. Supelco, Inc. Bellefonte. PA) is worth mentioning. When used with a thermal conductivity detector, the SP 1220 was shown to give a better resolution and a shorter elution time of VFA (12 min for C_1-C_6 and isomers) as compared to Resoflex® column packing (12-25 min for C_1-C_6 and isomers)[6]. The SP 1220 packing was recently shown to be useful for the efficient resolution of keto VFA[7].

An ideal chromatography method should involve both an efficient and rapid resolution of the components of a sample. The present communication deals with the improvement in the rapidity of a highly efficient GLC technique for VFA analysis[6] by oven temperature programmed conditions and using the SP 1220 column packing. This method is suitable for analyses with both thermal conductivity detector (TCD) and flame ionization detector (FID).

The 4 anaerobic bacterial strains used in this study included *Clostridium histolyticum* (C 22), *Propionibacterium acnes* (Pr 20), *Bacteroides asaccharolyticus* (B 5) and *Streptococcus morbillorum* (Sc 13) from our collection. All strains were inoculated, using the glove box procedure[8], in prereduced chopped meat carbohydrate medium (CMC, Carr Scarborough Microbiologicals Inc. GA) and the cultures were incubated for 48 h at 37 °C. The ether extraction procedure was similar to that described by Holdeman et al.[4]. Each pair of analyses with FID and with TCD was performed with the same ether extract. A Tracor (model MT 220) gas chromatograph was used throughout this study. The instrument was equipped with a FID, a TCD, a temperature programming system and dual column oven. It was paired to a recorder Tracor Westronics MT (span: 1 mV/25.5 cm) operated at 2.55 cm/min. 4 U-shaped glass columns (length: 183.0 cm, outer diameter: 0.64 cm, inner diameter: 0.4 cm) packed with 15% SP 1220/1% H_3PO_4 on 100-120 mesh Chromosorb W, acid washed (AW) were used. The SP 1220 stationary phase was obtained from Supelco Inc. Each column end was filled with phosphoric acid treated glass wool and conditioned for 72 h at 185 °C under a

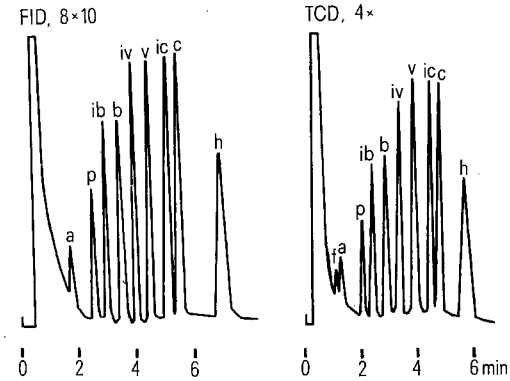

Fig. 1. Chromatograms of VFA standard mixture (1 meq. of each acid/100 cm^3) obtained when analysis conditions with FID and TCD were used. VFA: f, formic; a, acetic; p, propionic; ib, isobutyric; b, butyric; iv, isovaleric; v, valeric; ic, isocaproic; c, caproic; h, heptanoic.