CCXCIII. THE DISSOCIATING POWER OF SALTS OF FATTY ACIDS

PRELIMINARY PAPER

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This work had its origin in the observation that the dissociative change in colour induced in lobster shell by alcohol was partly reversible. This was made shortly before the appearance of the preliminary paper of Stern & Salomon [1937], who have drawn attention to the reversible colour change occurring upon mild heating of the chromoprotein, ovoverdin, from lobster eggs, and have explored part of this interesting field [1938]. Denaturation of ovoverdin, as ordinarily seen, involves the two processes (*a*) of dissociation of the astacin ester from the associated protein, giving the colour change green to red, and (*b*) of denaturation (with coagulation) of the protein itself. We deal here only with the dissociation, making the assumption that the colour change represents a change in the state of combination of the carotenoid with the protein. We found that this dissociation can be quickly induced at neutral reaction by traces of the Na salts of oleic and of other fatty acids, and further that these acids, when added to a respiring brain *brei*, inhibit respiration. It is suggested here that the two sets of observations are related, and that this inhibition is caused by an adsorptive dissociation of the prosthetic groups of certain enzymes from the apoenzymes.

Few investigations have been found of the general problem of the action of soaps upon enzymes. Velluz [1927] stated that fatty acids inhibit the action of pepsin, the effect increasing from C_6 to C_{18}; unsaturated fatty acids were more potent than the saturated ones, though with urease the latter were more active. Related to the question also seems to be the finding of Quastel [1932] that unsaturated glycerides increase the toxicity of the triphenylmethane series of dyes towards urease, though this action was rather specific.[1]

There is an evident relation also to the film penetration observations of Schulman & Rideal [1937] and Schulman [1937], though the nature of this relation is not yet defined.

Experimental

Ovoverdin. This was extracted from lobster eggs [Stern & Salomon, 1937] by grinding with sand and water and treatment of the fluid portion with an equal volume of saturated ammonium sulphate. After filtration, the ovoverdin was precipitated from the filtrate by more complete saturation with ammonium

[1] Upon completion of this work, there appeared a note in which Cook & Kreke [1938] state that fatty acids in a concentration of 0·5 mg./ml. stimulate the respiration of yeast, and at 1 mg./ml. stimulate skin respiration slightly. Higher concentrations were inhibitory. Such amounts cause massive depression in the pyruvate system here studied; a special further test showed that neither 0·1 mg. nor 0·05 mg. Na decoate had any stimulating effect with brain though these lie immediately below the concentration which has an inhibitory action, viz. 0·2 mg./3 ml.

sulphate, the precipitate collected upon a fluted filter paper, under cool conditions, and dissolved in 1% NaCl. Traces of ammonium sulphate were removed by dialysis against 1% NaCl. It was found to be important to keep the chromoprotein moist at all stages with dilute salt solution; drying or approximation to salt-free solutions rapidly causes the dissociative change from green to red. It is interesting in view of what follows that in its relation to salts and drying the chromoprotein follows closely in stability the pyruvate oxidase system in brain tissue, though there is a difference in sensitivity to freezing. This does not appear to affect ovoverdin, whereas it quickly destroys pyruvate oxidase.

Na salts of fatty acids. With the exception of the Na palmitate and oleate, which were of doubtful purity, the remaining soaps were obtained from commercial sources, but were subjected to several recrystallizations. The C_{10}, C_{12} and C_{14} acids were prepared from acids previously purified to the correct M.P. Some difficulty is experienced in obtaining solutions of the less soluble salts at the reaction required for test, approximately *p*H 7·4. It will be realized from the study of the *p*H relations at interfaces of the fatty acids [Peters, 1931; Wakelin, 1938] that at this reaction dodecoic acid tends to be present mainly as the acid and to leave an aqueous phase; decoate on the other hand shows a more marked water-solubility. Hence the use of a soap at this reaction must in reality mean the use of a mixture of salt and acid, and the really important point is to add it in a sufficiently soluble form to ensure that the mixture reaches the enzyme studied. In practice with dodecoate, tetradecoate and palmitate after solution in the appropriate amount of water, the whole was warmed, traces of phenol red added, and then HCl to give the desired *p*H. Where solution was possible only at 38°, the soap solution was kept in the warm bath until addition, together with a control containing water only. The soap and the water control were then added in similar volumes as a final addition to the Barcroft bottles. The highest concentrations used were Na octoate 0·1 *M*, Na decoate 0·045 *M*, Na dodecoate 0·047 *M* and Na tetradecoate 0·00206 *M*.

In other respects the respiration experiments with pigeon's brain tissue followed the usual technique of the laboratory; it will be recalled that the Ringer-phosphate medium (*p*H 7·3) does not contain Ca. The experimental volumes were 3·0 ml. in the Barcroft bottles; $1{\cdot}504 \times 10^{-3}$ *M* Na decoate = 1 mg. in 3·0 ml. In most experiments the figures quoted are the mean of duplicate estimations. The vitamin added was enough to produce maximum effects, 1·0–2·0 μg.; Na pyruvate, 6 mg. was added to each bottle.

Effect of Na salts upon ovoverdin

The minimum amount of Na salt necessary to cause dissociation was found by adding it in solution to 1·0 ml. of a suitable dilution of ovoverdin in 1% NaCl or in phosphate buffer (*p*H 6·7), until the green colour had disappeared. Table I shows the results with buffer present; in absence of the latter, the results were 20% higher but in the same sense.

Table I. *Amounts of Na salts of fatty acids required to cause dissociation of dilute ovoverdin solutions. Temp. 16–20°*

	$\times 10^{-3}$ *M*
Na octoate	>12·8
Na decoate	4·17
Na dodecoate	1·105
Na tetradecoate	1·042
Na palmitate	<1·2
Na oleate	1·09

The optimum concentration is reached with C_{12}; the difference between this and C_{14} is not significant. The figure for palmitate is unreliable owing to the low solubility. Since the change from water-solubility to lipoid-solubility takes place with a length of C chain of about 10, it seems to be clear that the effect is correlated with adsorption at the surface of an internal phase, and is explained if the acid displaces the astacin ester from its combination.

Reversibility. We may ask whether the acid interferes by combining irreversibly, or whether the effect is a loose displacement. It was not found possible to extract the fatty acid by any means which would leave the protein undenatured; but a simple addition of $CaCl_2$ to 0·02–0·04 *M* readily causes precipitation of such fatty acids under these conditions, and in the absence of phosphate will reverse the colour change to green. This is quite consistent with the idea that we are dealing with a reversible adsorptive phenomenon.

Effects upon some oxidation enzyme systems

The above observations are of interest in themselves, but they gain in importance by the finding that a similar action takes place upon a brain *brei*. The question has not been thoroughly explored; only with pyruvate and succinate as substrates has it been investigated, and with the latter not in detail. The interesting point is that with pyruvate there is inhibition increasing from the C_8 acid to the C_{12}, as with the dissociation of the lobster pigment. Fig. 1 illustrates the action of sodium decoate. In Table II the results for pyruvate are collected under the headings of the acid to which they refer. Where the actions of more than one acid were compared upon the same brain this may be seen from the no. of the exp. (column 6, Table II). In general the results for different exps. seemed to be sufficiently similar to make this the best method of examination. In the summary (Table II), the essential point is made that the inhibitory action increases from C_8 to C_{12}; after this the results for tetradecoate are of the same order, but are slightly complicated by solubility. Palmitate seems to be less active but the result is again rather uncertain, and the figure for concentration can only be regarded as a maximum one. With decoate and dodecoate amounts of 0·2–0·3 mg. produce definite effects. It is suggested in explanation that the acids displace an essential component of the oxidase system, as with the model ovoverdin. The experiments have been done throughout upon the avitaminous brain in order to obtain possible extra information; they are not complicated by the possibility that time was not allowed for combination of vitamin, because at least 5 min. were allowed after addition of vitamin and before adding the fatty acid. In many cases the inhibition for vitamin is greater than that for pyruvate alone; but this is probably not significant, because the figure for respiration without added vitamin has a relatively larger contribution from the residual respiration, which appears to be less inhibited.

In support of the idea that some coenzyme may be displaced from combination by the acid, we have found that with $4{\cdot}51 \times 10^{-3}$ *M* decoate the CO_2 evolution from pyruvate by washed yeast (acetone-treated) with added cocarboxylase is reduced by 35% both alone and in the presence of 10 μg. vitamin B_1; this experiment was carried out as described by Ochoa & Peters [1938].

The oxidase system for succinate in brain is more stable than that for pyruvate; the former survives freezing and extraction with distilled water. Similarly it is not much affected by fatty acid; a complete experiment upon this is given in exp. 12 with dodecoate; similar results have been obtained with decoate.

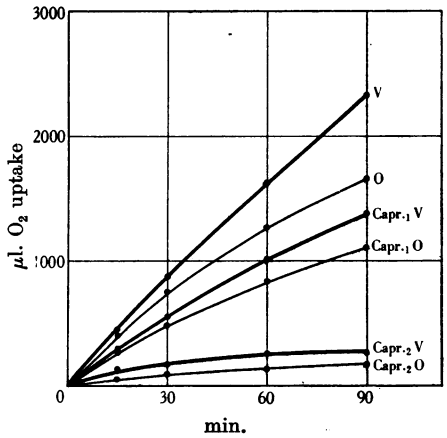

Fig. 1. The effect of small amounts of Na decoate upon the O_2 uptake of avitaminous pigeon brain in Ringer-phosphate pyruvate medium. Upper curves: V = with and O = without vitamin. Middle curve (capr.$_1$), addition of 0·2 mg. decoate. Lower curves (capr.$_2$), 0·7 mg. decoate.

Table II. *Inhibiting effect of Na salts of fatty acids upon rate of the O_2 uptake of pigeon's brain tissue (in presence and absence of vitamin B_1) with pyruvate, expressed as a decrease in μl./g./hr. and as a % decrease.*

	Conc. $M \times 10^{-3}$	No vitamin		Vitamin		Exp.
		μl.	%	μl.	%	
Octoate	16·0	−905	64·5	−1575	71·6	5
	4·8	−256	18·0	− 851	38·8	5
	1·9	+ 39	Nil	− 357	18·4	6
	1·6	+ 43	Nil	− 265	12·0	5
Decoate	5·27	−848	88·7	−1586	94·1	1
		−832	92·6	−1375	94·1	2
	4·51	−646	82·5	−1314	89·5	3
	1·5	−520	40·6	−1182	55·4	6
		−485	48·5	−1007	59·0	7
	1·05	−282	14·6	− 621	42·5	2
	0·31	− 3	Nil	− 210	14·4	2
		− 32	4·0	− 207	14·0	3
Dodecoate	4·94	−753	83·9	−1262	82·9	2
	1·65	−774	77·4	−1341	78·6	7
	0·99	−325	38·5	− 674	43·6	8
	0·424	−100	11·9	− 312	20·2	8
Tetradecoate	0·67	−211	31·2	− 516	33·6	10
	0·13	− 57	8·5	− 315	20·4	10
	0·33	− 28	3·5	− 175	13·3	11
	0·065	−115	15·0	− 160	12·1	11
Palmitate	0·6	− 79	10·6	− 226	15·8	9
	0·12	+ 18	Nil	− 102	7·2	9
Oleate	1·97	−578	60·5	−1217	72·2	1
	0·77	−523	54·7	− 917	54·4	1

The periods of respiration after start of the exp. upon which the above figures are based are as follows: 30–60 min., exps. 5, 7; 30–90 min., exps. 1, 2, 3, 6, 9; 30–120 min., exps. 8, 10, 11.

Summary

Acid	Conc. $M \times 10^{-3}$	Decrease % O	Decrease % V
C_{12}	1·65	77·4	78·6
C_{10}	1·5	48·5	59·0
C_8	1·6	Nil	12·0

Exp. 12. Avitaminous brain used so as to eliminate pyruvate oxidation to some extent in the residual oxidation. The figures quoted are for the period of respiration 15–90 min. NaD = Na dodecoate.

Substances present	Rate of resp. μl./g./hr.	Difference
No addition	968	
Succinate	2566	1598
$0{\cdot}705 \times 10^{-3}$ *M* NaD	470	
Same + Succ.	1922	1452
$1{\cdot}41 \times 10^{-3}$ *M* NaD	323	
Same + Succ.	1662	1339

Na succinate 0·082 *M*. Note that $1{\cdot}65 \times 10^{-3}$ *M* reduces respiration with pyruvate 77 % and 0·99, 38 %. The decrease in the residual oxidation was here 52 and 66 % respectively.

As would be expected from this, the succinic dehydrogenase from liver (pigeon) showed no reduction in activity upon adding Na dodecoate ($3{\cdot}4 \times 10^{-3}$ *M*). On the other hand exp. 13 shows that the system which causes dehydrogenation of pyruvate in brain is much affected.

Exp. 13. *Inhibition of dehydrogenase action by Na dodecoate.* The tissue from two normal pigeon brains was thoroughly mashed and extracted 6 times with 7 ml. 1 % KCl and 4 times with 7 ml. Ringer phosphate, *p*H 7·4. Care was taken to grind the whole thoroughly with a glass rod and to keep ice cold throughout but not frozen. Thunberg tubes were set up containing 50 mg. tissue, 0·2 ml. methylene blue (1:5000), 3 mg. Na pyruvate and 1·0 ml. Ringer-phosphate. The Na pyruvate was placed in the stopper and added after evacuation just before placing in the bath at 38°. One tube contained in addition 1 mg. Na dodecoate. Figures are quoted for one out of four similar experiments.

	Time for decoloration
Control	30 min.
Na pyruvate	9 min.
Na pyruvate with Na dodecoate	>150 min.

The results for the pyruvate dehydrogenase of pigeon's brain confirm those of Lipmann [1937]. It will be seen that the dodecoate has inhibited not only the pyruvate effect but also the residual reduction.

It might be thought that some of the phenomena described above were merely the result of the well-known cytolysing action of soaps, and consequent destruction of enzyme action intimately bound up with cell structure. This clearly could not apply to the ovoverdin, nor to the acetone-treated yeast; in the case of the pyruvate oxidase system, Dixon & Meyer [1936] have shown that many brain cells are destroyed in forming a *brei*; in confirmation and extension of this Dr Carleton has found that, in our brain *brei*, the cytoplasmic outlines of the cells are destroyed (unpublished). Hence the evidence is that here too the effect is on the enzyme system itself rather than the cell.

Discussion

There seems to be no doubt as to the practical importance of these observations. Where there is a possibility that fatty acids may pre-exist or be formed by lipase action or otherwise during the course of experimental procedure, it seems certain that they will influence adversely experimental results with isolated systems. In particular it is likely that they constitute some of the difference between the tissue *brei* and the slice. The insolubility at *p*H 5·5–6·0 of the inhibitor of brain glycolysis described by Geiger [1938] suggests an effect of this type. Again, this may be part of the reason why Quastel & Wheatley [1933],

and Jowett & Quastel [1935] found oxidation of fatty acids by slices of liver, but none with *brei*. If there is substance in the idea that the behaviour of ovoverdin serves as a model for the more loosely associated groups of enzymes, a new experimental tool is indicated. It has been pointed out previously [Peters, 1931] that the fact that fatty acids of the water-insoluble type ionize over the physiological range of *p*H is of biological significance. Here we find that part of the colloidal phase of a chromoprotein in solution is affected by the same type of acid. This shows that such interfaces exist in practice in such solutions. Further it is clear that the cell possesses yet another simple method of controlling its activities.

SUMMARY

Small amounts of the Na salts of saturated fatty acids (C_{10}, C_{12}, C_{14}) cause dissociation of the astacin ester from the protein compound in ovoverdin; this change can be reversed by Ca salts.

Similarly these soaps exert a markedly inhibitory action upon the pyruvate oxidase system; the effect upon the succinoxidase system is much less.

It is suggested that these phenomena are related.

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