

1950

Fritz Lipmann, 1948

The Rockefeller University

Follow this and additional works at: <https://digitalcommons.rockefeller.edu/harvey-lectures>

Recommended Citation

The Rockefeller University, "Fritz Lipmann, 1948" (1950). *Harvey Society Lectures*. 53.
<https://digitalcommons.rockefeller.edu/harvey-lectures/53>

This Book is brought to you for free and open access by Digital Commons @ RU. It has been accepted for inclusion in Harvey Society Lectures by an authorized administrator of Digital Commons @ RU. For more information, please contact nilovao@rockefeller.edu.

BIOSYNTHETIC MECHANISMS*

FRITZ LIPMANN

*Professor of Biological Chemistry, Harvard Medical School, and
Head, Biochemical Research Laboratory
Massachusetts General Hospital
Boston, Massachusetts*

IT seems that in the field of biosynthesis we have a rare example of progress leading to simplification. Not long ago the mechanisms involved here appeared almost hopelessly complex. Meanwhile a better understanding of the pathways of metabolic energy turnover helped much to open the gates. The realization that the living organism tends to use a standard type of chemical energy, phosphate bond energy, in metabolic syntheses¹ reduced greatly the apparent complexity. The use of the standard energy presupposes a certain uniformity of processing.

When we look at the great variety of cell constituents it seems an overwhelming task to find the processes by which all these compounds are manufactured. However, if we start to focus our attention on primary linkages, we discover that the links in different classes of compounds are quite similar. As an endlessly repeating process in the building up of cell material, we find elimination of water between groupings. One of the most common reactants in these condensations through intermolecular dehydration is the carboxyl group. Quite generally, ester and peptidic links appear to be formed by way of carboxyl activation. The energy input necessary to perform these linkings as they occur in fat, protein and elsewhere is very similar, around 3,000 calories.²

Phosphate bonding is used as mentioned in the metabolic machinery as a means to parcel and transfer energy. Available energy is processed initially into energy-rich phosphate bonds of approximately 15,000 calories each and distributed through the adenylic-adenyl pyrophosphate and probably other systems. A key, or at least one of the keys, to its very general applicability appears to be the acid anhydride nature of the energy-rich phosphate bond. This

* Lecture delivered December 16, 1948.

makes it possible for the ATP to act as a common reagent for all these condensations involving the removal of water. In those reactions involving the carboxyl groups, this general statement can be qualified more precisely. It appears that here the primary reaction is the formation of an acid anhydride between carboxyl and phosphoric acid. The scope of such carboxyl activation as preliminary was widened by the observation that it appears also frequently to operate in carbon to carbon condensation³ leading to the building up of larger carbon structures. The following picture has been used to tentatively illustrate the operation of phosphate bond energy in protein and fat synthesis:¹

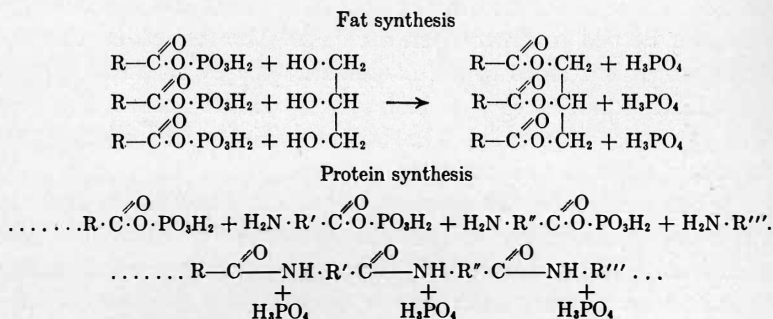
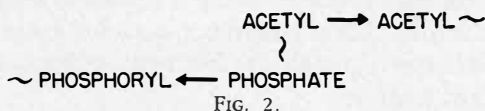


FIG. 1. Tentative scheme of fat and protein synthesis (1941).

Quite accidentally, I was started on the problem of carboxyl activation during the study of a partial reaction in carbohydrate oxidation; namely, the oxidation of pyruvate.^{4, 5} In certain microbial systems, indications appeared that a compound of the constitution of acetyl phosphate was elaborated, an anhydride of acetic and phosphoric acid.^{6, 7} Eventually we proceeded to the isolation of acetyl phosphate by following up the initial observation that pyruvate oxidation yielded energy-rich phosphate bonds which could transfer to adenylic acid.^{8, 9} This was at the time of some importance since it represented a fairly well understood mechanism by which a respiratory reaction yielded energy-rich phosphate bonds. The observation foreshadowed a generality of phosphate bond generation in energy metabolism.

At first it seemed that we had here just another member of the

group of phosphate compounds first observed in fermentation reactions. Of greater importance, however, than this donation of phosphate into the energy-rich phosphate pool appeared the possibility that acetyl phosphate might be able to make its organic part available for synthesis. Like the head of Janus, this molecule appeared to be able to face in two directions:



To document the phosphate transfer reaction, an old experiment is presented in Table I showing the transfer of phosphate from acetyl phosphate to adenylic acid to form ATP. The early

TABLE I
TRANSFER OF ACETYL-BOUND PHOSPHORUS TO ADENYLIC ACID^a

All data are given in micromoles. Fifteen micromoles of synthetic acetyl phosphate were added and incubation was in nitrogen only.

INCUBATION TIME	ADENYLIC ACID ADDED	P ₇	P _{ae}	P _i
At start		2.1	15.0	4.0
After 60 min.	15.6	8.0	2.9	10.0
After 60 min.	0	2.1	6.9	12.1

P₇ is equal to P_i hydrolyzed seven minutes in N HCL at 100°. P_{no} equals Acetyl-P as determined by the first method of Lipmann and Tuttle.⁸ P₁ is equal to inorganic P.

experiment⁹ suggested reversibility of the reaction between acetate and the adenylic acid system. This phosphate transfer reaction is very widespread in many microorganisms^{9, 10, 11} and lately also has been observed in liver extracts.¹² However, when we started to approach the more interesting problem of acetyl donation, we met with considerable difficulties.

A large number of trial experiments with acetyl phosphate were carried out which never were published because they gave uniformly negative results. Acetoacetate synthesis and citric acid synthesis were tried in tissue homogenates, but with negative results. More encouraging results obtained in some bacterial systems concerned

the reversibility of the so-called phosphoroclastic reaction; the split of pyruvate to acetyl phosphate and either formate or carbon dioxide and hydrogen was studied. Particularly, the synthesis of pyruvate from formate and an acetyl precursor was easily established using isotopic carbon containing formate.¹³ Furthermore, the recombination of formate with acetate could be shown by using isotopic acetate but this reaction occurred to an appreciable extent only on addition of ATP as phosphate donor. (Table II.) Addition of synthetic acetyl phosphate, however, seemed not to affect the resynthesis of pyruvate.

TABLE II
NECESSITY OF ADENYLPYROPHOSPHATE FOR
CONDENSATION OF ACETIC-FORMIC TO PYRUVIC ACID¹³

$\text{CH}_3\text{C}^{13}\text{OOH}$ with 5.6 per cent excess C^{13} was incubated with pyruvate, formate and with and without adenylypyrophosphate, in *E. coli* extract.

C^{13} EXCESS IN CARBONYL OF PYRUVATE	ADENYLPYROPHOSPHATE
0.03	—
0.15	+
0.24	+

I had now become thoroughly interested in acetyl activation and for further information turned then to study acetylation of aromatic amines. Analytically, this was an agreeable system since a very sensitive method was available for determination of sulfonamides. After some trial and error, it was found¹⁵ that the pigeon liver is distinguished by yielding homogenates and extracts uncommonly active in acetylation. However, when synthetic acetyl phosphate was tried as acetyl donor in this system, it proved inactive. The result was somewhat obscured through the presence of a powerful acetyl phosphatase¹⁶ in such tissue extracts which rapidly destroys acetyl phosphate. But the activity of this homogenate should have been great enough to give at least some acetylation. This result then confirmed again the inactivity of synthetic acetyl phosphate. Likewise, acetylation of choline was tried with acetyl phosphate in brain homogenate and with negative results.

At the same time, however, Nachmansohn, who had been studying the acetylation of choline in the course of studies on nerve

activity, was less preoccupied with acetyl phosphate and followed more the general idea that phosphate bond energy should be operating in acetylation.^{17, 18} He added acetate and adenyl pyrophosphate together to brain extracts and found that with this combination vigorous acetylation occurred. When the effect of acetate + ATP was tried in the acetylation system of aromatic amines an excellent acetylation was obtained.¹⁵ In Table III acetylation of sulfanilamide in liver homogenate is shown with ATP plus acetate as acetyl donor.*

TABLE III

ANAEROBIC CONJUGATION THROUGH ADENYL PYROPHOSPHATE¹⁵

Samples of 1 ml. of homogenate, with 0.03 M sodium bicarbonate, were incubated at 37° in Warburg vessels filled with N₂ - 5 per cent CO₂ which had been passed over heated copper oxide. All vessels contained acetate in 0.02 M final concentration and 90 γ of sulfanilamide were added at the start with the sodium fluoride. The adenyl pyrophosphate was added from two separate annexes in equal portions, the first at the start and the second after 15 minutes incubation. The total incubation time was 30 minutes.

EXPERIMENT NO.	NaF	ADENYL PYROPHOSPHATE		SULFANILAMIDE CONJUGATED γ	REMARKS
		ADDED	REMAINING		
	mole per l.	mg. P _i	mg. P _i		
1	0.08	0.32	0.16	49	Fresh homogenate
	0.08	0	0	9	
	0.02	0.32	0.09	37	
	0.02	0	0	5	
2	0.02	0.32	0.08	57	Same, after freezing overnight
3	0.02	0	0	15	Different Homogenate
	0.02	0.32	0	28	

* The apparent inactivity of acetyl phosphate in situations where ATP plus acetate showed activity made us for some time weigh the possibility of an ATP-acetate reaction product which in some way differs from synthetic acetyl phosphate. Attempts, however, to identify an ATP-acetate reaction product different from acetyl phosphate failed consistently. In various bacterial extracts, particularly in such of *E. coli*, a rather abundant formation of such product obtains. The isolation of this product led eventually always to a compound indistinguishable from synthetic acetyl phosphate.

It furthermore appears now^{20, 42} that if certain microbial protein fractions are added, synthetic acetyl phosphate donates acetyl in animal tissue extracts for sulfanilamide acetylation as well as for all other coenzyme A-linked reactions. The factor responsible for the activation appears to be identical with the fraction catalyzing a Co A-dependent enzymatic equilibration of (*Cont'd. on page 104*)

THE COENZYME FOR ACETYLATION

During the study of sulfonamide acetylation in pigeon liver extract unexpectedly a good lead was obtained towards a solution of the puzzling problem of metabolic acetate utilization. It appeared that a coenzyme was involved in this process.¹⁵ On dialysis as well as on aging, the enzyme solution lost the ability to acetylate, which was regained on addition of boiled extracts (Table IX). None of the known coenzymes could replace this factor and the isolation of this apparently new coenzyme was therefore attempted.

TABLE IV
REVERSIBLE INACTIVATION OF THE ACETYLATION SYSTEM
THROUGH DIALYSIS OR AUTOLYSIS¹⁵

The pigeon liver extract, treated or untreated, was incubated in open narrow test tubes in a water bath of 37°. The tubes contained 1 ml. of extract in a total volume of 2 ml.; magnesium chloride and sodium acetate were present in 0.02 M concentration. The extract was combined with the activator, and, after adaptation to the bath temperature, the experiment was started through addition of a mixture of 0.32 mg. of adenylyl polyphosphate P, 88γ of sulfanilamide, and fluoride to 0.05 M final concentration.

TREATMENT OF EXTRACT	FILTRATE OF BOILED ORGAN ADDED, CORRES. TO GM. FRESH WEIGHT	SULFA- NILAMIDE CONJUGATED	INCUBATION TIME
Untreated		γ	min.
Kept 16 hrs., 7°		69	65
		7	40
Dialyzed 16 hrs., 7°	0.2 gm. rat liver	58	
	0.2 gm. rat liver	0	65
		42	
Untreated		59	50
Kept 16 hrs., 7-10°		0	
	0.4 gm. rat liver	28	
	0.4 gm. pigeon breast muscle	14	

On purification it appeared that the new coenzyme, coenzyme A, was a pantothenic acid derivative.²² The same coenzyme was found to activate the acetylation of choline.²³ Concurrently, Nachmansohn

(Continued from p. 103) acetyl-bound phosphate with inorganic phosphate.^{19, 21} Such equilibration seems possible only through the intermediacy of a catalyst which temporarily binds the acetyl in energy-rich linkage. The acetyl-charged Co A-enzyme complex is then most likely the immediate acetyl donor.

and Behrman²⁴ and Feldberg and Mann²⁵ observed the need for an activator in choline acetylation. We find the activators of Nachmansohn and of Feldberg and Mann to be identical with coenzyme A. The equivalence of a purified coenzyme preparation for acetylation in brain of choline and in liver of sulfanilamide, respectively, is shown in Figure 3.

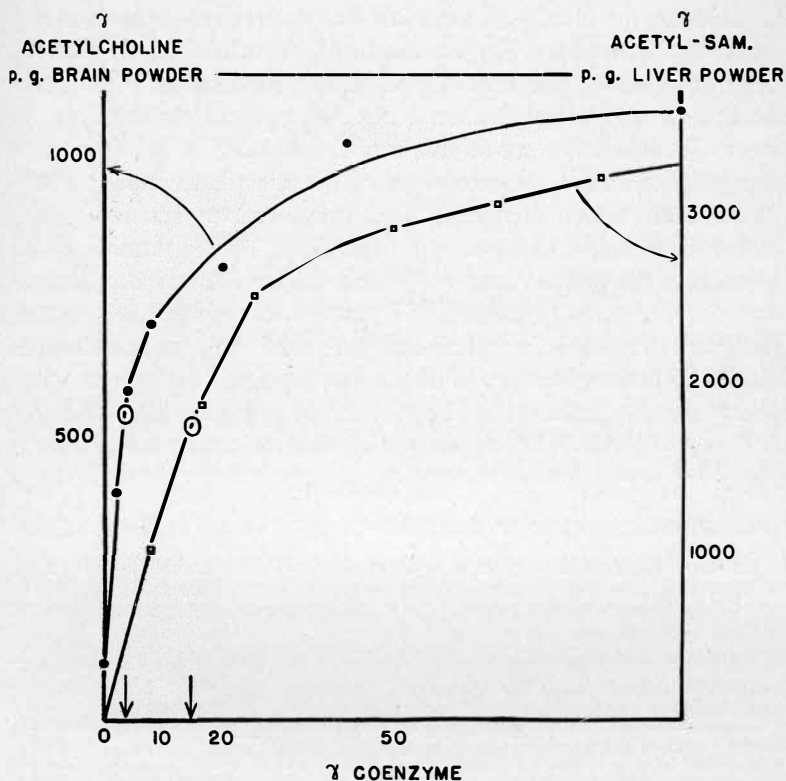


FIG. 3. Comparison of the effect of the same purified coenzyme preparation on aged pigeon brain extract for choline acetylation and aged pigeon liver extract for sulfonamide acetylation.

HYDROXYLAMINE AS TRAPPING REAGENT FOR ACTIVE CARBOXYL GROUPS

During the work on the acetyl problem, considerable use has been made of the peculiar reactivity of hydroxylamine with acti-

vated carboxyl groups. I would like to insert here some comments on this very useful reaction. Tuttle and I,²⁶ while working with acetyl phosphate, noticed that on addition of hydroxylamine, phosphate was split off almost instantaneously in a nonenzymatic reaction and hydroxamic acid formed. The well-known strong purple color which hydroxamic acid gives with iron in acid solution could be used for quantitative determination of acetyl phosphate or any other acyl phosphate. By substituting the earlier rather tedious determination by differential phosphate precipitation,¹⁷ this easy method of acyl determination quickened progress in the field. A most convenient feature of this newer method²⁶ is that it is quite independent on the presence of inorganic phosphate or other phosphate esters which frequently had prevented an accurate determination of acetyl phosphate. Furthermore, hydroxylamine could be used as an acyl interceptor.²⁷ This device allowed the demonstration of a primary acetate-ATP reaction in pigeon liver extract, yielding presumably acetyl phosphate which was trapped immediately by hydroxylamine. Without the trapping reagent, no acetyl phosphate accumulation had been observed in pigeon liver extract.* In Table V, the ATP dependent hydroxamic acid formation is

TABLE V

PHOSPHORYLATION OF ACETATE IN PIGEON LIVER EXTRACTS²⁷

0.5 ml. of pigeon liver extract (1 g. of acetone-dried liver extracted with 10 ml. of 0.02 M bicarbonate solution and centrifuged) was incubated for 60 min. at 37° in test tubes. The final volume was 1 ml. A.. samples contained 0.02 M neutralized hydroxylamine hydrochloride.

Acetate, millimole	10	3	1	—	10
Labile adenylpyrophosphorus, millimole	10	10	10	10	—
Hydroxamic acid millimole	1.76	1.77	1.35	0.90	0.38

shown. The great affinity of acetate in this enzyme system is noteworthy. Most significant is the participation of coenzyme A in this acetate activation.

More explicitly, the effect of coenzyme A on hydroxamic acid

* It is now found that the microbial enzyme which facilitates utilization of acetyl phosphate in tissue extracts²⁰ also catalyzes, in reverse, the accumulation of acetyl phosphate from ATP plus acetate in pigeon liver extracts.

formation is shown in Figure 4 and compared with sulfonamide acetylation in the same extract.

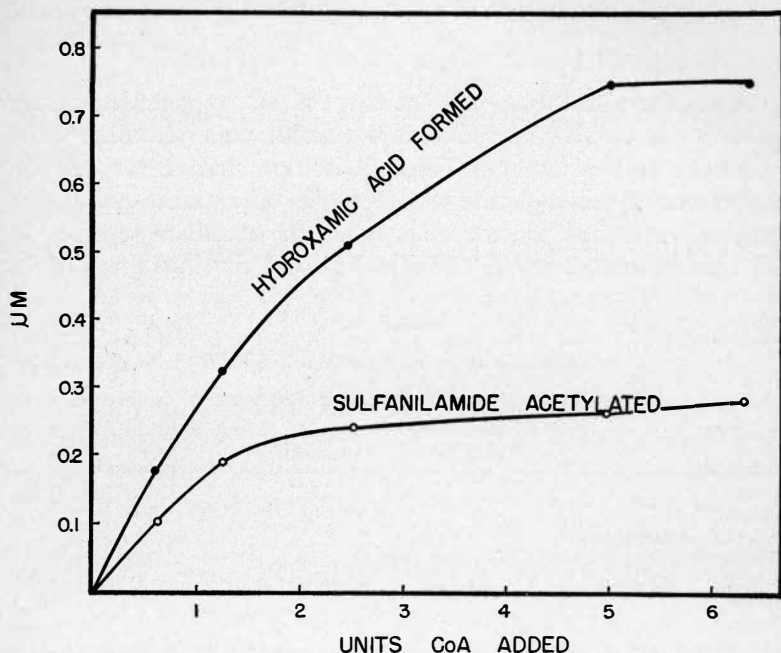


FIG. 4. Comparison of coenzyme A effect on ATP-dependent hydroxamic acid formation from acetate and of sulfonamide acetylation carried out in parallel in the same pigeon liver extract.

Recently, hydroxylamine has shown its more general usefulness as an interceptor of activated carboxyl groups in the study of glutamine synthesis. Like acetylation, glutamine synthesis is an ATP-dependent reaction. This was demonstrated by Speck²⁸ in pigeon liver extracts similar to the one used by us for acetylation experiments and by Elliott²⁹ in brain extracts. The study of this reaction is facilitated by the possibility of substituting hydroxylamine for ammonia and measuring colorimetrically the glutam-hydroxamic acid formed. The similarity of the enzymatic mechanism of sulfonamide acetylation and glutamination is not too surprising since in both cases we are dealing with the formation of a peptidic bond.² So far, however, it has not been possible to dem-

onstrate a participation of coenzyme A in glutamine synthesis. It might be that the pantothenic acid containing coenzyme is specifically designed for the handling of acetyl transfer reactions.

THE MAJOR FUNCTION OF COENZYME A

A tie of acetate activation to coenzyme A, i.e., to pantothenic acid, proved a productive help to a more intimate understanding of the metabolic role of acetate. Through isotope studies,^{43, 44} the involvement of this molecule was shown in an amazing number of synthetic reactions. On the other hand, the abundant presence in all cells of coenzyme A³⁰ (Table VI) and the likelihood of its

TABLE VI
COENZYME A IN ANIMAL TISSUES³⁰

All values are given in units of coenzyme A per gm. of fresh tissue

	HUMAN	RABBIT	RAT	PIGEON
Liver		112	132	105
Adrenal		65	91	
Adrenal demedullated			79	
Kidney		50	74	
Brain		40 (Cortex)	28	40
Heart		26	42	45
Testes		26		
Intestine			26	
Thymus			20	
Skeletal muscle		6		
Blood plasma	0			
Red blood cells	3-4			

equivalence with intracellular pantothenic acid³¹ indicated a major metabolic function for this coenzyme. Its function in acetylation, helpful as it had been for its recognition, could only be a minor side issue. The study of this reaction, however, had given the suggestion of a common precursor in a variety of reactions involving the two-carbon residue.

Acetoacetate Synthesis: Pyruvate synthesis, a first example of carbon-carbon linking through carboxyl activation was previously discussed. As shown by the following equations, in acetoacetate like pyruvate synthesis we seemed to deal with an intermolecular dephosphorylation without energy loss. If just the energy residing

in the phosphate bond were used for the linking of the two carbons and inorganic phosphate were split out:*

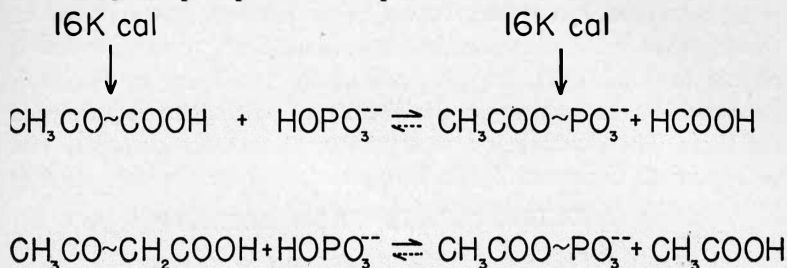


FIG. 5. A comparison of the phosphoroclastic split of pyruvate and acetoacetate and the reverse synthesis of the respective keto acids. The dotting of the arrow in the reverse direction shall indicate that the reversal of the phosphoroclastic split is most likely not due to a straight reversal reaction.

An early rough calculation¹⁶ of the energy needed for the linking of two acetate molecules to acetoacetate indicates a value of around 16,000 calories which closely matches the energy available from the acyl phosphate bond.

TABLE VII
ENZYMATIC CONDENSATION OF ACETATE TO
ACETOACETATE IN LIVER EXTRACTS³

A 1 ml. purified fraction† was used per sample (corresponding to 180 mg. of dry liver powder) in a total volume of 3 ml. containing 0.01 M cysteine, 0.1 M sodium bicarbonate, and other additions as indicated; incubated for two hours at 38°.

	0.02 M ACETATE, 0.01 M ATP, Co A, 40 UNITS PER ML.	SAME, BUT NO CO A	SAME, BUT NO ATP	SAME, BUT NO ACETATE
Acetone, micromoles	3.2, 3.3	0.1	0.2	0.3

* Subsequent experiments, however, using acetyl phosphate marked with C₁₄ in the carboxyl group,²⁰ in the presence of a large excess of inactive acetate, have shown this scheme to be incomplete. Pigeon liver fractions were used in combination with the microbial (*Clostridium kluyveri*) enzyme mediating acetyl phosphate utilization. On enzymatic synthesis, the C₁₄ of the acetyl phosphate carboxyl appeared equally in both the carbonyl and carboxyl parts of acetoacetate. The double labelling of acetoacetate must mean that two molecules of acetyl phosphate are brought into reaction to form acetoacetate. Phosphate balance studies, furthermore, have shown that two acetyl phosphates are split with the condensation of each acetoacetate.

† Between 40 and 70 per cent ammonium sulfate saturation.

Recently Soodak and I³ could show that such a reaction really occurs. In the same pigeon liver extract which had furnished already so many synthetic enzyme systems, a formation of acetoacetate was found when ATP plus acetate was added. Such an experiment is shown in Table VII. It was particularly gratifying to find this condensation belonging to the family of coenzyme A catalyzed reactions. The dependence on coenzyme A is more clearly shown in Figure 6. Concurrently Stadtman and Barker³² in their studies

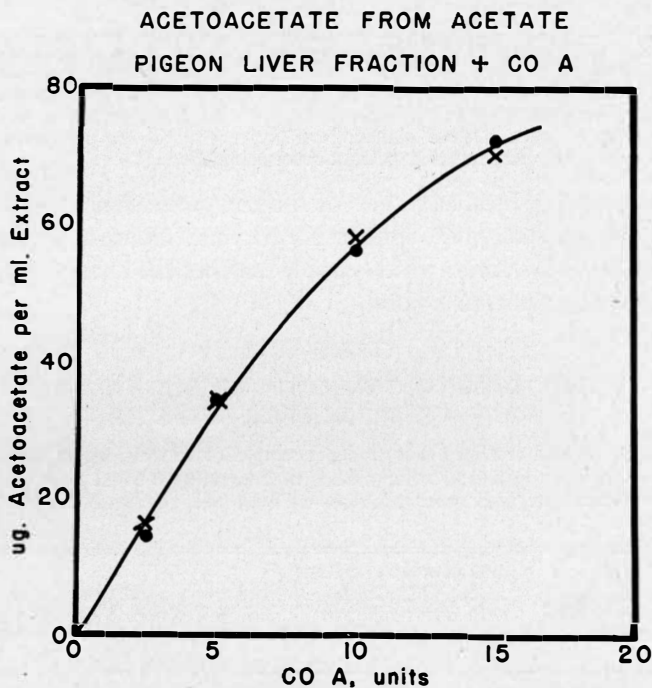


FIG. 6. Dependence of acetoacetate synthesis on coenzyme A in pigeon liver extract. The protein fraction between 40 and 70 per cent ammonium sulfate saturation was used for this experiment.

on fatty acid synthesis demonstrated the reverse reaction, the phosphoroclastic split of acetoacetate to acetyl phosphate and acetate in extracts of *Clostridium kluyverii*.

The acetoacetate condensation deserves attention because it represents a rather fundamental metabolic reaction. It is most likely

that an analogous condensation occurs primarily in many cases where acetate serves as a building block for larger molecules. In Figure 7 we try to focus attention on some outstanding features of this "head to tail" reaction between two acetates: carboxyl, or

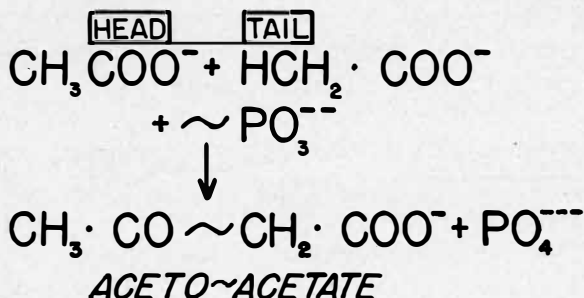


FIG. 7.

head, activation by primary reaction with energy-rich phosphate carries the energy which remains stored in the $-\text{COCH}_2-$ link. The energetic equivalence of $-\text{CO} \sim \text{CH}_2-$ and $-\text{CO} \sim \text{OPO}_3$ is noteworthy. It is not quite clear yet if, like in citrate synthesis, the methyl or tail part in the other acetate needs also to be activated.*

A distinction of head and tail activation of acetate has proven to us quite useful for a classification of condensations involving acetate. On these grounds we distinguish three types of reactions¹ a head reaction as is encountered in plain acetylation of amino groups, or of aromatic amines,¹⁵ histamine,³³ and of choline²⁴,² a head and tail reaction as it is found in acetoacetate condensation,³ and a plain tail reaction as it occurs in citrate synthesis. The latter is schematically pictured in Figure 8. A common factor in all these reactions is participation of coenzyme A.

Citric Acid Synthesis: The evidence that citric acid synthesis belonged into this group had for some time remained indirect, deriving from experiments with pantothenic acid deficient organisms. Quite early Teague and Williams³⁴ suspected a participation of this vitamin in carbohydrate metabolism. This view was strengthened by the subsequent work of Dorfmann et al³⁵ and of Hills³⁶ demonstrating with *Proteus morgani* a participation by pantothenic acid in the process of pyruvate oxidation. No further decision regard-

* Cf. footnote p. 109.

ing the point of action was however possible then and for some time this observation remained somewhat of an interesting curiosity. The participation of the pantothenic acid derivative coenzyme A in enzymatic acetylation and the wide distribution of this coenzyme

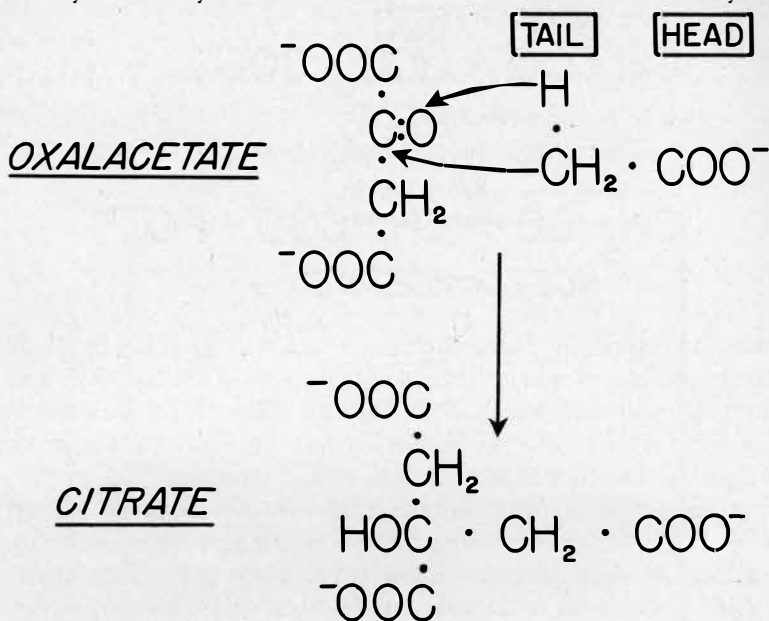


FIG. 8.

prompted us to seek as common factor the acetyl activation, to carry pyruvate into the citric acid cycle. Hills' work already was suggestive of such interpretation.

Novelli and I³⁷ first used Dorfmann's organism to establish the expected correlation between coenzyme A content and pyruvate oxidation. The results are shown in Table VIII. Confirming and

TABLE VIII
COMPARISON OF THE EFFECT OF ADDED PANTOTHENIC ACID ON
RESPIRATION AND ON COENZYME A CONTENT³⁷

	Q_{O_2}	COENZYME UNITS PER G. DRY WEIGHT
+ Pyruvate	5.9	130
+ Pyruvate and pantothenate	10.2	520

extending these observations, Olson and Kaplan³⁸ then, with liver slices, found a quite astonishing parallel between the rate of pyruvate utilization and coenzyme A levels. The results of their experiments under various conditions of pantothenic acid deficiency and recovery are summarized in Figure 9. The proportionality line pointing towards the origin intimates that in the absence of coenzyme A no pyruvate would be metabolized by the liver. In both cases, however, a further narrowing down towards the precise point of action met at first with difficulty.

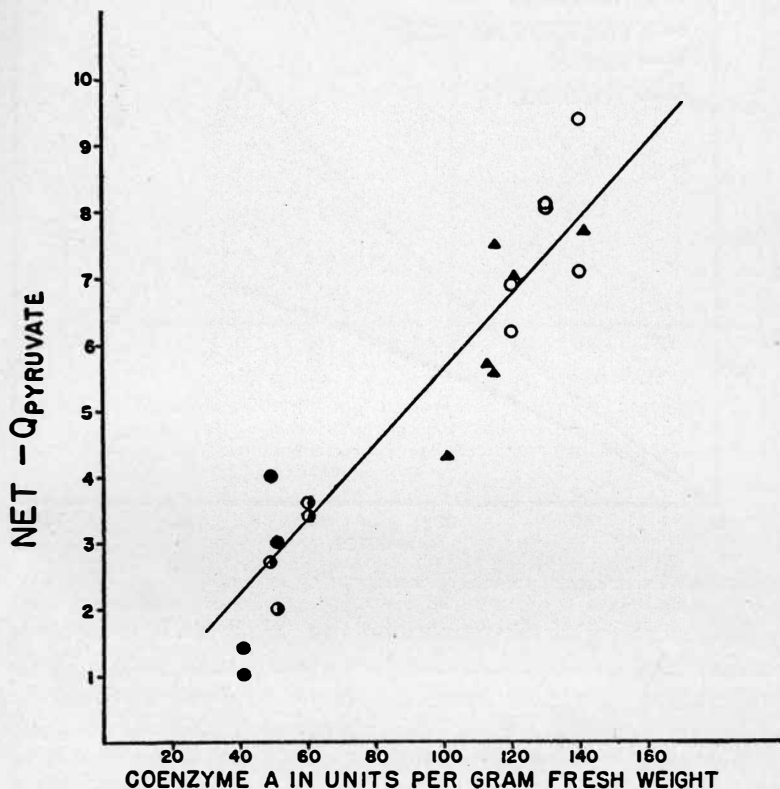


FIG. 9. Dependence of pyruvate utilization in duck liver slices on coenzyme A content. The following symbols represent the various groups of ducks: deficient (●), deficient treated in vitro (⊖), deficient treated in vivo by intraperitoneal injection of 10 mg. of calcium pantothenate per 100 gm. of body weight one to two hours before observation (⊥), and normal controls fed ad libitum (○).

We therefore turned our attention to an organism with an outspoken acetate metabolism, choosing a strain of yeast that metabolizes acetate rather rapidly.³⁹ Earlier the use of isotopic acetate had shown that in yeast the acetate is metabolized through the citric acid cycle.⁴⁰ Yeast thus appeared to be a very suitable organism for

EFFECT OF CoA ON ACETATE OXIDATION IN YEAST

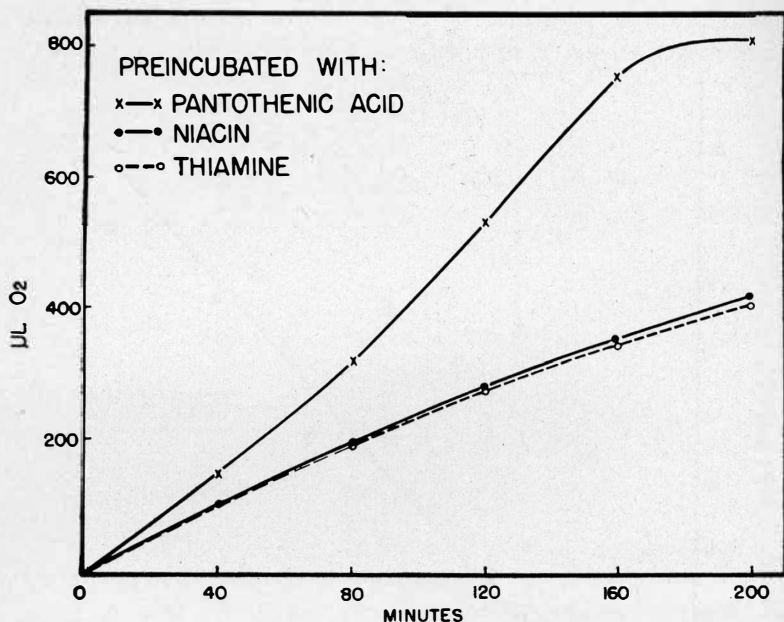


FIG. 10. Experimental conditions corresponding to those given in Table XIV.

a further testing of the proposition that coenzyme A is involved in citric acid condensation. Comparable samples of yeast, respectively rich or poor in coenzyme A, were prepared and tested for the ability to metabolize acetate. The rate of respiration was found to follow closely the coenzyme A level (Fig. 10). Even more convincingly, the need of coenzyme A for the respiratory attack on acetate is shown in experiments with ethanol as substrate. Here respiration and acetate accumulation were followed at the same time. It will be seen in Figure 11 that with enough coenzyme A

EFFECT OF CoA ON ALCOHOL OXIDATION IN YEAST

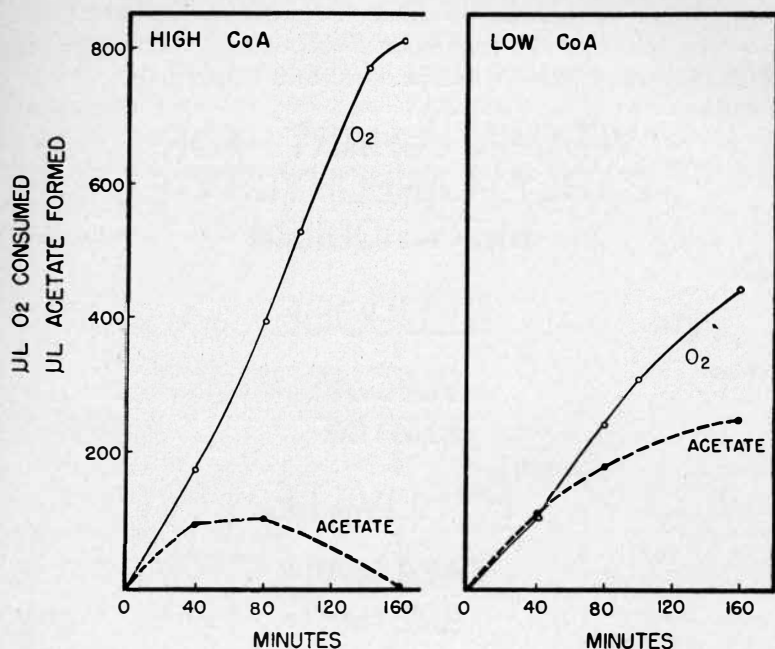


FIG. 11. Comparison of the effect of coenzyme A on oxygen consumption and acetate accumulation with ethanol as a substrate. The acetate formation was followed by the enzymatic micromethod for acetate determination of Soodak and Lipmann, *Fed. Proc.*, 7:190 (1948).

TABLE IX

EFFECT OF COENZYME A ON GLUCOSE RESPIRATION

Each vessel contained 4.2 mg. yeast in 3 ml. M/30 primary potassium phosphate containing 5 μ M glucose. The yeast was originally grown in the presence of a minimum amount of pantothenic acid and pretreated for one hour in a glucose phosphate medium with and without pantothenic acid. The coenzyme data refer to the content after pretreatment with and without pantothenic acid, the higher value referring to the pantothenic acid-treated sample. The recorded experiment was carried out in Warburg vessels at 37° over a period of 80 minutes. Acetate was determined by the enzymatic method described by Soodak and Lipmann.

COENZYME A	370 u/mg.	135 u/mg.
Oxygen, μ M	-15.9	-11.7
Acetate, μ M accumulated	0	+ 2.5

only a small and temporary accumulation of acetate occurs in the early part of the experiment. With the coenzyme-deficient yeast, however, although the respiration is depressed, acetate is piling up steadily; the ethanol oxidation stops at the acetate stage.

VITAMIN CHART FOR EARLY CARBOHYDRATE METABOLISM

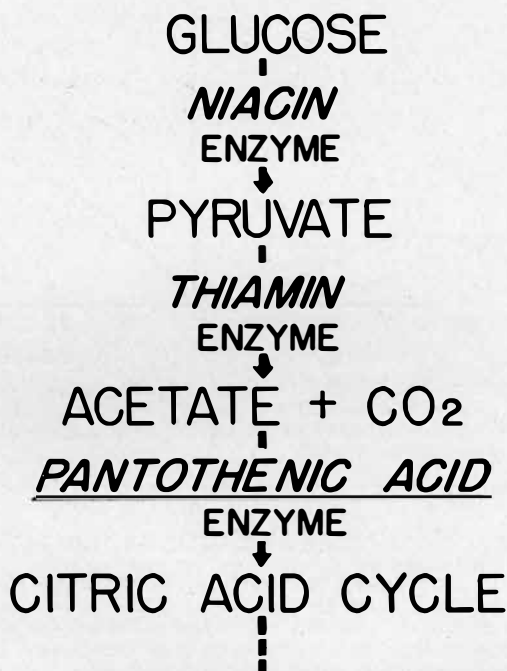


FIG. 12.

Using glucose as the substrate, a deficiency in coenzyme A likewise resulted in accumulation of acetate as shown in Table IX. The oxygen consumption, however, as would be expected was relatively less depressed. This observation confirms Weinhouse's isotope experiments⁴⁰ which indicated that the yeast oxidizes glucose by way of acetate and through the citric acid cycle.

Assessing the function of pantothenic acid in the main stretch of carbohydrate oxidation, and including the other functionally identified vitamins, the following scheme obtains:

It appears that for every one of the three principal metabolic steps to carry one-half molecule of glucose into the citric acid mill a special catalyst had to be elaborated; pyridine nucleotide for the first dehydrogenation, cocarboxylase for the first decarboxylation coupled with the second dehydrogenation and coenzyme A for condensation of the two-carbon fragment with oxaloacetic acid.*

TABLE X

CITRIC ACID SYNTHESIS FROM ACETYL PHOSPHATE⁴²

1.0 cc of dialyzed *E. coli* extract in final volume of 2.5 cc; all tubes contained 0.025 M oxaloacetate; pH 8.0; incubated one hour at 30°. Additions were present in the following final concentrations: acetate, 0.05 M; ATP, 0.02 M; lithium acetyl phosphate, 0.004 M; Co A, 17 units.

	ADDITIONS		CITRIC ACID SYNTHESIZED
			$\mu\text{M}/\text{cc}$ Extract
Acetate	ATP	—	0.23
—	ATP	Co A	0.50
Acetate	ATP	Co A	1.32
Acetyl phosphate		—	0.25
Acetyl phosphate		Co A	4.0
Acetyl phosphate + acetate		Co A	3.9

In pigeon liver extract complemented by bacterial enzymes, citric acid synthesis has now also been demonstrated with acetyl phosphate-oxaloacetate.⁴²

SYNOPSIS

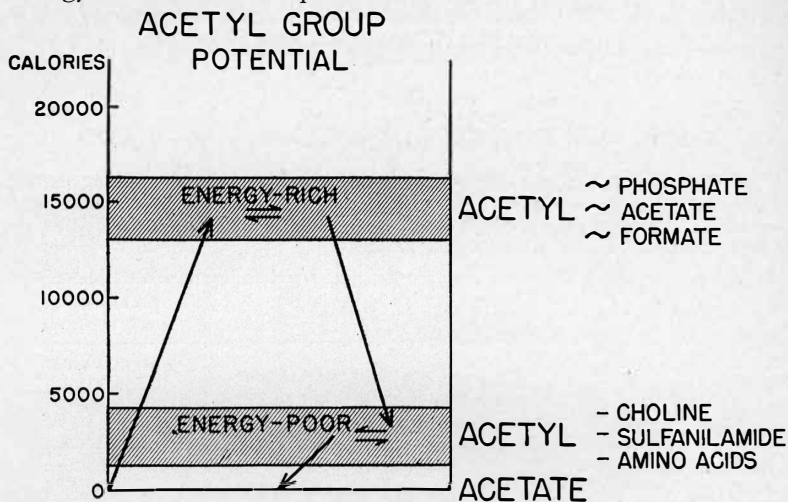
In the preceding section mechanisms were described which yield

* Since the delivery of this lecture, our round-about evidence for a participation of coenzyme A in citric acid synthesis has been confirmed in isolated enzyme systems. Using our ammonium sulfate fractions of pigeon liver extract used previously³ for acetoacetate condensation, Stern and Ochoa⁴¹ tested for citrate formation from oxaloacetate, acetate and ATP. They found considerable reactivity but only on addition of coenzyme A. Significantly their tests indicate that citrate and not iso-citric or aconitic acid are formed as the primary condensation product.

Concurrently, experiments by Novelli in our laboratory⁴² gave analogous results with extracts of *E. coli* and of yeast. In the *E. coli* extracts, somewhat to our surprise, synthetic acetyl phosphate proved rather superior to ATP + acetate as acetyl precursor. The reaction was found to be dependent on coenzyme A.

acetyl donors by an activation of acetate ions, from the base level. Such activation occurs by reaction with ATP wherewith the acetyl residue is lifted to the 15,000 calorie level. In Figure 13, the relationship is pictured between acetyl phosphate and various other compounds where the acetyl radical appears metabolically.

We see that acetyl may either be carried back and forth on the energy-rich level, or drop down to the lower level of an ester or



peptide bond. In the latter case, a good deal of the energy of the original acetyl compound is degraded. It appears rather significant that these acetyl levels are analogous to the phosphate levels described earlier.¹ The energy-rich acetyl in its various forms is equivalent to the energy-rich phosphates. The ester or amino linked acetyl, however, is on the level of energy-poor phosphate esters; the acetate and inorganic phosphate ions represent respectively the base levels in the group potential scale.

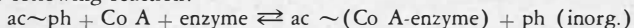
The energetic possibility of an acetyl shift between acetyl phosphate, acetoacetate and pyruvate is confirmed through the synthesis of pyruvate and similarly of acetoacetate from acetyl phosphate or ATP + acetate. There is a considerable amount of evidence, particularly from isotope experiments^{43, 44} that a direct acetyl derivation may occur from pyruvate and acetoacetate. Therefore the possibility

is to be considered that from these keto acids the acetyl radical may be transferred without intermediation of phosphate. The observations on an interchange-ability of sucrose and glucose-1-phosphate as source of active glucose may serve as an example of this type of direct group exchange from an organic link.⁴⁵ A certain indication for such an occurrence may be taken from tracer experiments on the exchange between acetyl and inorganic phosphate.¹⁹ Such an exchange occurs more or less rapidly in extracts of *Lactobacillus delbrueckii*, *Clostridium butylicum* and *E. coli*, most rapidly in *Clostridium butylicum* where within a few minutes a complete exchange may be observed. This exchange, although studied preliminarily only in crude extract, takes place without and is not influenced by addition of acceptors which intermediately would carry the phosphate through a cycle.

In parallel with the reasoning of Doudoroff, Barker and Hassid⁴⁵ it might be assumed that such exchange indicates an intermediate acetyl-enzyme compound derived by reaction with acetyl~phosphate or with acetyl~acetate or acetyl~formate (pyruvate) which could likewise donate ~acetyl to the enzyme. The preservation of the energy excess in the acetyl residue by reaction with either a protein or coenzyme would then explain a direct utilizability of the acetyl of pyruvate and acetoacetate for further reactions. It is quite tempting to speculate on coenzyme A functioning as acetyl carrier.* Such acetyl transfer system would help to bring order into the presently rather confusing isotope data concerning acetate and acetate precursors.⁴⁴

It is most likely anyway that coenzyme A operates in all processes involving acetate or energy-rich acetyl for synthesis of larger structures. Figure 14 charts the two main metabolic branches which have their rooting in the C₂ residue. We find that we are reasonably well informed about some of the primary reactions on both sides. As such we consider the acetoacetate and citric acid type of condensa-

* This interpretation has gained considerable support through further observations on the exchange between acetyl-bound and inorganic phosphate showing it to be mediated by a coenzyme A-dependent enzyme system.²¹ This indicates then the following reaction:



The acetyl-Co A-enzyme complex appears at present to be the nearest approximation towards the chemical identification of the so-called active C₂ residue.

tion. The primary linking of C_2 residue in porphyrins and purines remains still to be explored further. Nevertheless, one of the more interesting results of the observations reported here appears to be a somewhat surer footing we are obtaining in the field of biosynthe-

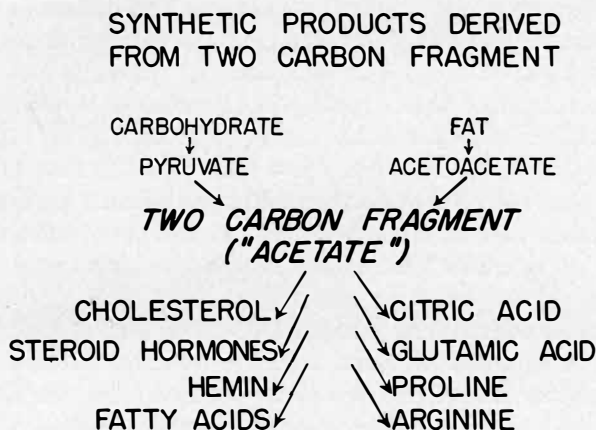


FIG. 14.

sis of larger carbon structures. Specifically so far only enzymatic synthesis of small molecules was accomplished. We understand how two acetyls are linked to acetoacetate and furthermore Stadtman and Barker⁴⁶ found with preparations of *Cl. kluyveri* enzymatic synthesis from acetyl phosphate-hydrogen of the 6-carbon-chain of hexanoic acid. It appears therefore reasonable to assume that the long chains of stearic, palmitic and other fatty acids are built by lining up of acetyls followed by reduction. This gives us a mechanism of straight carbon chain formation.

The lining up of the same molecule to long straight chains⁴⁷ appears to be, although a basic, a rather primitive biosynthetic reaction. We might call this a one-dimensional synthesis. However, straight fatty acids, as well as folded and branched steroids, appear to originate from the same acetyl building block. In the case of the steroid, however, we are dealing with at least a two-dimensional synthesis and the problem naturally becomes more involved.* A juxtaposition of steroid and fatty acids shows clearly the additional

* Citric acid synthesis may be viewed as a simple example of an implantation of an acetyl branch onto a straight carbon chain.

structuration which has to go into the steroid synthesis (Fig. 15). We are here dealing with a dominant biosynthetic problem, the forming of specific two- or three-dimensional patterns from equal or similar units.

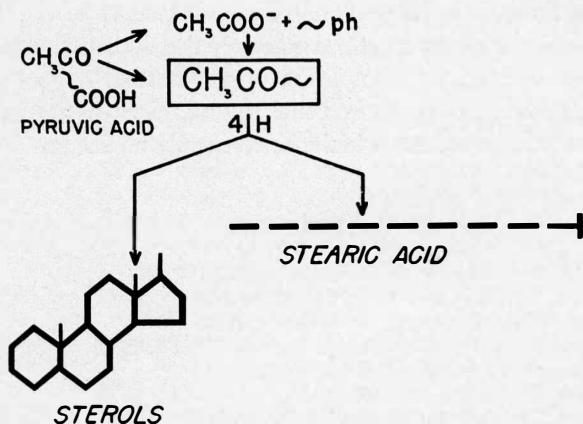


FIG. 15.

Particular attention has been focused on the problem not only of production but of constant reproduction of the same involved structure. A tendency seems to have developed to assume a spatial master pattern or template which serves as mold for structure reproduction. It may be that some super-structuration can be imposed in such a manner on preformed structures. The present experience, although on lower and intermediate levels of complexity, seems not to favor spatial or mold-like arrangement but rather a building up of structures through a one-by-one succession of attachments, first probably to straight chains with subsequent branching⁴⁷ and cross linking. It seems to us more likely that macromolecular patterns are mostly woven rather than stamped, to use a comparison to human procedures fulfilling the analogous functions of pattern production and reproduction.

It was with some surprise that I discovered that considerations of a very similar type arose in the quite remote field of nerve physiology. In Adrian's book⁴⁸ on the conduction of nerve impulses, a noteworthy discussion of the problem of visual structure recognition is found. Here likewise a decision is sought as to whether tem-

poral or spatial mechanisms are operating in the eye-brain combinations: using the triangle as example, the structural recognition of such structure may involve (1) a template or space image deposited somewhere in the brain or (2) a dynamic process of eye movement around the three cornered circumference of the triangle. Present evidence seems to prefer strongly the dynamic or temporal mechanism of structure recognition by movement. It goes to show the general overlapping, to find that in biological disciplines so far apart there arise problems which are so closely analogous and similarly solved.

REFERENCES

1. LIPMANN, F.: *Advances in Enzymology*, 1:99, 1941.
2. LIPMANN, F.: *Federation Proc.*, 9: (in press).
3. SOODAK, M. and LIPMANN, F.: *J. Biol. Chem.*, 175:999, 1948.
4. LIPMANN, F.: *Skandinav. Arch. f. Physiol.*, 76:266, 1937.
5. LIPMANN, F.: *Enzymologia*, 4:65, 1937.
6. LIPMANN, F.: *Nature*, 144:381, 1939.
7. LIPMANN, F.: *Cold Spring Harbor Symposia on Quantitative Biol.*, 7:248, 1939.
8. LIPMANN, F. and TUTTLE, L. C.: *J. Biol. Chem.*, 153:571, 1944.
9. LIPMANN, F.: *J. Biol. Chem.*, 155:55, 1944.
10. KOEPESELL, H. J.; JOHNSON, M. J.; and, MEEK, J. S.: *J. Biol. Chem.*, 154:535, 1944.
11. UTTER, M. F. and WERKMAN, C. H.: *Arch. Biochem.*, 5:413, 1944.
12. KAPLAN, N. O. and LIPMANN, F.: *Federation Proc.*, 7:163, 1948.
13. UTTER, M. F.; LIPMANN, F.; and, WERKMAN, C. H.: *J. Biol. Chem.*, 158:521, 1945.
14. STRECKER, H. and WOOD, H. G.: *Federation Proc.*, 8:257, 1949.
15. LIPMANN, F.: *J. Biol. Chem.*, 160:173, 1945.
16. LIPMANN, F.: *Advances in Enzymology*, 6:231, 1946.
17. NACHMANSOHN, W. and MACHADO, A. L.: *J. Neurophysiol.*, 6:397, 1943.
18. NACHMANSOHN, D. and JOHN, H. M.: *J. Biol. Chem.*, 158:157, 1945.
19. LIPMANN, F. and TUTTLE, L. C.: *J. Biol. Chem.*, 158:505, 1945.
20. CHOU, T. C.; NOVELLI, G. D.; STADTMAN, E. R.; and, LIPMANN, F.: *Federation Proc.*, 9: 1950.
21. STADTMAN, E. R.: *Federation Proc.*, 9: 1950.
22. LIPMANN, F.; KAPLAN, N. O.; NOVELLI, G. D.; TUTTLE, L. C.; and, GUILLARD, B. M.: *J. Biol. Chem.*, 167:869, 1947.
23. LIPMANN, F. and KAPLAN, N. O.: *J. Biol. Chem.*, 162:743, 1946.
24. NACHMANSOHN, D. and BERMAN, M.: *J. Biol. Chem.*, 165:551, 1946.
25. FELDBERG, W. and MANN, T.: *J. Physiol.*, 104:411, 1946.
26. LIPMANN, F. and TUTTLE, L. C.: *J. Biol. Chem.*, 159:21, 1945.
27. LIPMANN, F. and TUTTLE, L. C.: *J. Biol. Chem.*, 161:415, 1945.
28. SPECK, J. F.: *J. Biol. Chem.*, 168:403, 1947.
29. ELLIOTT, W. H.: *Biochem. J.*, 42:5, 1948.

30. KAPLAN, N. O. and LIPMANN, F.: *J. Biol. Chem.*, 174:37, 1948.
31. NOVELLI, G. D.; KAPLAN, N. O.; and LIPMANN, F.: *J. Biol. Chem.*, 177:97, 1949.
32. STADTMAN, E. R. and BARKER, H. A.: *J. Biol. Chem.*, 174:1039, 1948.
33. ROSENTHAL, S. M.; MILLICAN, R. C.; and, TABOR, H.: Private communication.
34. TEAGUE, P. C. and WILLIAMS, R. J.: *J. Gen. Physiol.*, 25:777, 1942.
35. DORFMAN, A.; BERKMAN, S.; and, KOSER, S. A.: *J. Biol. Chem.*, 144:393, 1942.
36. HILLS, G. M.: *Biochem. J.*, 37:418, 1943.
37. NOVELLI, G. D. and LIPMANN, F.: *Arch. Biochem.*, 14:23, 1947.
38. OLSON, R. E. and KAPLAN, N. O.: *J. Biol. Chem.*, 175:515, 1948.
39. NOVELLI, G. D. and LIPMANN, F.: *J. Biol. Chem.*, 171:833, 1947.
NOVELLI, G. D. and LIPMANN, F.: *Federation Proc.*, 7:177, 1948.
40. WEINHOUSE, S. and MILLINGTON, R. H.: *J. Am. Chem. Soc.*, 69:3089, 1947.
41. STERN, T. R. and OCHOA, S.: *J. Biol. Chem.*, 179:491, 1949.
42. NOVELLI, G. D. and LIPMANN, F.: *J. Biol. Chem.*, 182:213, 1950.
43. BLOCH, C.: *Physiol. Rev.*, 27:574, 1947.
44. GURIN, S. and CRANDALL, D. I.: *Cold Spring Harbor Symposia on quantitative Biol.*, 13:118, 1948.
45. DOUDOROFF, M.; BARKER, H. A.; and, HASSID, W. Z.: *J. Biol. Chem.*, 168:725, 1947.
46. STADTMAN, E. R. and BARKER, H. A.: *J. Biol. Chem.*, 180:1117, 1949.
47. CORI, G. T. and CORI, C. F.: *J. Biol. Chem.*, 151:57, 1943.
48. ADRIAN, E. D.: *The Physical Background of Perception*, Oxford, Clarendon Press, 1947.