Development of the Acetylation Problem, a Personal Account*

Fritz Lipmann

Biochemical Research Laboratory, Massachusetts General Hospital and
Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts

THE fact that my Swedish colleagues have honored me with the Nobel prize gives me some confidence to consider my own effort more seriously as a part in the general effort of biochemistry of today. I therefore thought of tracing, in the segment of my interest, the recent development of facts and ideas which led, it seems, to a fuller understanding of the chemical functioning of the organism. When I started out in the middle twenties, biochemistry was just trying to break away from the major concern with breakdown processes and procedures. With the slowly increasing comprehension of biosynthetic mechanisms, a rather radical change of attitude ensued which is, I feel, not quite fully realized even at the present time. Out of the early, justifiably stubborn empiricism grew up a definite rational structure. Process patterns emerged and it became important to recognize certain rules and introduce new terms, thereby emphasizing the fact that biochemistry was now developing into an adult science, best characterized, maybe, as organismic technology.

In my development, the recognition of facts and the rationalization of these facts into a unified picture, have interplayed continuously. After my apprenticeship with Otto Meyerhof, a first interest on my own became the phenomenon we call the Pasteur effect, this peculiar depression of the wasteful fermentation in the respiring cell. By looking for a chemical explanation of this economy measure on the cellular level, I was prompted into a study of the mechanism of pyruvic acid oxidation, since it is at the pyruvic stage that respiration branches off from fermentation. For this study I chose as a promising system a relatively simple-looking pyruvic acid oxidation enzyme in a certain strain of *Lactobacillus delbrueckii* (*1*). The decision to explore this particular reaction started me on a rather continuous journey into partly virgin territory to meet with some unexpected discoveries but also to encounter quite a few nagging disappointments.

Discovery of Acetyl Phosphate

The most important event during this whole period, I now feel, was the accidental observation that, in the *L. delbrueckii* system, pyruvic acid oxidation was completely dependent on the presence of inorganic phosphate. This observation was made in the course of attempts to replace oxygen by methylene blue. To measure the methylene blue reduction manometrically, I had to switch to a bicarbonate buffer instead of the otherwise routinely used phosphate. In bicarbonate, to my surprise, pyruvate oxidation was very slow, but the addition of a little phosphate caused a remarkable increase in rate (Fig. 1). Figure 2 shows the phosphate effect more drastically. It represents use of a preparation from which all phosphate was removed by washing with acetate buffer. Then it appeared that the reaction was really fully dependent on phosphate.

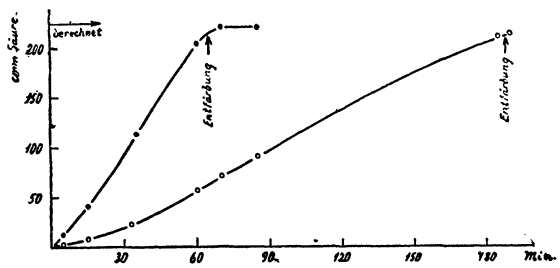


Fig. 1. Effect of phosphate on acid formation during the reduction of methylene blue. ●——● represents enough phosphate added to make a total concentration of 4×10^{-3} mole. ○——○ represents no phosphate.

In spite of such a phosphate dependence, the phosphate balance measured by the ordinary Fiske-Subbarow procedure did not at first indicate any phosphorylative step. Nevertheless, the suspicion remained that phosphate in some manner was entering into the reaction and that a phosphorylated intermediary was formed. As a first approximation, a coupling of this pyruvate oxidation with adenylic acid phosphorylation was attempted. And, indeed, addition of adenylic acid to the pyruvic oxidation system brought out a net disappearance of inorganic phosphate, accounted for as adenosine triphosphate (Table 1). In parallel with the then just developing fermentation picture, I concluded that the missing link in the reaction chain was acetyl phosphate. In partial confirmation it was shown that a crude preparation of acetyl phosphate, synthesized by the old method of Kämmerer and Carius (*2*) would transfer phosphate to adenylic acid (Table 2). However, it still took quite some time from then on to identify acetyl phosphate definitely as the initial product of pyruvic oxidation in this system (*3, 4*). Most im-

* This paper is based on Dr. Lipmann's Nobel prize address delivered in Stockholm, Sweden, 11 Dec. 1953 when the 1953 award in medicine and physiology was made. It is published with permission of the Nobel Foundation and will also appear in *Les Prix Nobel 1953*.

Table 1. Disappearance of inorganic phosphate with adenylic acid.

	Initial value	0.125*M* pyruvate	0.125*M* pyruvate 0.03*M* adenylic acid	0.03*M* adenylic acid
Inorganic P (mg)	0.59	0.53	*0.31*	0.59
Easily hydrolized P (mg)	0	0.06	*0.28*	0.01
O_2 (μlit)		490	474	58

portant during this and later work was the development of procedures (*5*) and in particular of the very handy hydroxamic acid method (*6*) for the determination of acyl phosphates and other reactive acyl derivatives.

At the time these observations were made, about a dozen years ago, there was, to say the least, a tendency to believe that phosphorylation was rather specifically coupled with the glycolytic reaction. Here, however, we had found a coupling of phosphorylation with a respiratory system. This observation immediately suggested a rather sweeping biochemical significance, of transformations of electron transfer potential, respiratory *or* fermentative, to phosphate bond energy and therefrom to a wide range of biosynthetic reactions (*7*).

There was a further unusual feature in this pyruvate oxidation system in that the product emerging from the process not only carried an energy-rich phosphoryl radical such as was already known, but the acetyl phosphate was even more impressive through its energy-rich acetyl. It rather naturally became a contender for the role of "active" acetate, for the widespread existence of which the isotope experience had already furnished extensive evidence. I became, therefore, quite attracted by the possibility that acetyl phosphate could serve two rather different purposes, either to transfer its phosphoryl group into the phosphate pool, or to supply its active acetyl for biosynthesis of carbon structures. Thus acetyl phosphate should be able to serve as acetyl donor as well as phosphoryl donor, transferring, as is shown in Fig. 3, on each side of the oxygen center, such as indicated by Bentley's early experiments on cleavage of acetyl phosphate in H_2O^{18} (*7a*).

Table 2. Transfer of phosphate from acetyl phosphate to adenylic acid with bacterial preparations. Fresh solution, containing 0.75 mg acid-labile P in 0.5 ml, 46 mg dry bacteria, total volume 1.25 ml, with 0.04*M* in NaF.

Adenylic acid (mg)	4	0.1	
Inorganic P, inorg. + labile (acetyl P) (mg)	1.12	1.39	1.37
P, after 7 min hydrolysis at 100°C in normal HCl (mg)	1.47	1.48	1.47
P_7 formed (mg)	0.35	0.09	0.10

These two novel aspects of the energy problem, namely (i) the emergence of an energy-rich phosphate bond from a purely respiratory reaction, and (ii) the presumed derivation of a metabolic building-block through this same reaction, prompted me not only to propose the generalization of the phosphate bond as a versatile energy-distributing system but also to aim from there toward a general concept of transfer of activated groupings by carrier as the fundamental reaction in biosynthesis (*8, 9*). Although in the related manner the appearance of acetyl phosphate as a metabolic intermediary first focused attention to possible mechanisms for the metabolic elaboration of group activation, it soon turned out that the relationship between acetyl phosphate and acetyl transfer was much more complicated than anticipated.

Since a better understanding of the mechanisms of group activation seemed to become a most urgent problem in biosynthesis, I now set out to find a suitable system to check on the assumption that acetyl phosphate represented active acetate. After working out a relatively easy method to prepare the compound (*5, 10*), a first unexpected difficulty arose when it appeared that animal tissues contain rather generally a very active, specific, and heat-stable acetyl phosphatase (*9, 11*). In crude preparations of muscle, liver, and other tissues, the half-life of acetyl phosphate is only a few minutes. This strange activity in animal

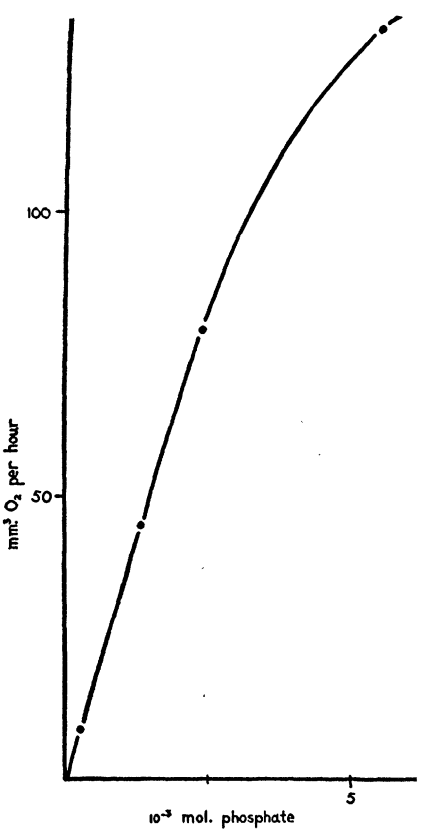


Fig. 2. Phosphate-dependence of pyruvate oxidation.

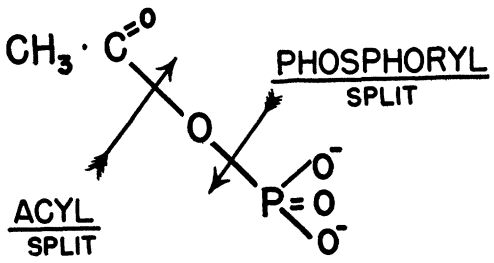


Fig. 3. Acetyl phosphate.

tissues made tests with this substance very difficult.

In looking for a sensitive method to study acetyl transfer, the acetylation of aromatic amines was chosen eventually as a most promising and technically easy procedure. We were, furthermore, quite confident that any results obtained with this method could be generalized over the whole metabolic territory concerning the transfer of active acetate, including such reactions as citrate, acetoacetate, and lipid synthesis. Acetylation of sulfonamide had been found to occur in rabbit liver slices (*12, 13*). However, for our purposes, we had to eliminate cell membrane barriers to test for the activity of complex intermediary metabolites. Although acetylation was found with rabbit liver homogenate, the reaction was rather weak. In search of a more active system, pigeon liver homogenate was tried and found to harbor an exceedingly potent acetylation system (*11, 12*). This finding of a particularly active acetylation reaction in cell-free pigeon liver preparations was most fortunate and played a quite important part in the development of the acetylation problem.

We had eventually arrived at the point where the desired test for acetyl phosphate as an acetyl precursor could be performed. Although the acetyl phosphatase activity of the pigeon liver homogenate was considerable and, to some extent, obscured the test with acetyl phosphate, it became, nevertheless, clear to us that in this preparation, acetyl phosphate did not furnish active acetate (*11*). Under anaerobic conditions the massive concentrations of acetyl phosphate, no acetyl groups for the acetylation of sulfonamide could be derived under conditions where an easy acetylation occurred with a respiring homogenate.

It furthermore appeared that, as an energy source, the particle-bound oxidative phosphorylation of the kind observed first by Herman Kalckar (*14*) could be replaced by ATP, as had first been observed with the acetylation of choline in brain preparations by Nachmansohn and his group (*15, 16*). Using ATP and acetate as precursors, it was possible to set up a homogenous particle-free acetylation system obtained by extraction of acetone pigeon liver. In this extract likewise acetyl phosphate was unable to replace the ATP-acetate as acetyl precursor.

Discovery and Identification of Coenzyme A

In spite of this disappointment with acetyl phosphate, our decision to turn to a study of acetylation started then to be rewarding in another way. During these studies we became aware of the participation of a heat-stable factor which disappeared from our enzyme extracts on aging or dialysis. This cofactor was present in boiled extracts of all organs as well as in microorganisms and yeast. It could not be replaced by any other known cofactor. Therefore, it was suspected that we were dealing with a new coenzyme. From then on, for a number of years, the isolation and identification of this coenzyme became the prominent task of our laboratory. The problem now increased in volume, and it was my good fortune that a group of exceedingly able people were attracted to the laboratory; first Constance Tuttle, then Nathan O. Kaplan, and shortly afterward G. David Novelli. More recently, Morris Soodak and John Gregory and quite a few others have made here most important contributions to the advance of this problem.

Table 3. Reversible inactivation through dialysis or autolysis; 1 ml of extract in a total volume of 2 ml; magnesium chloride and sodium acetate were present in 0.02*M* concentration. The experiment was started through addition of a mixture of 0.32 mg of adenyl polyphosphate P, 88 μg of sulfanilamide, and fluoride to 0.05*M* final concentration.

Treatment of extract	Filtrate of boiled organ added, corresponding to fresh weight	Sulfanilamide conjugated (μg)	Incubation time (min)
Untreated		69	65
Kept 16 hr, 7°C		7	40
	0.2 g rat liver	58	
Dialyzed 16 hr, 7°C		0	65
	0.2 g rat liver	42	
Untreated		59	50
Kept 16 hr, 7° to 10°C		0	
	0.4 g rat liver	28	

Early data on the replacement of this heat-stable factor by boiled extracts are shown in Table 3. The pigeon liver acetylation system proved to be a very convenient assay system for the new coenzyme (*17*), since on aging for 4 hr at room temperature, the cofactor was completely autolyzed. Fortunately, on the other hand, the enzyme responsible for the decomposition of this factor was quite unstable and faded out during the aging, while the acetylation apoenzymes were unaffected.

Figure 4 shows coenzyme A (CoA) assay curves obtained with acetone pigeon liver extract. Finding pig liver a good source for the coenzyme, we set out to collect a reasonably large quantity of a highly purified preparation and then to concentrate on the chemistry with this material. In this analysis we paid particular attention to the possibility of finding in this obviously novel cofactor one of the vitamins, then not as yet metabolically identified. In this task we

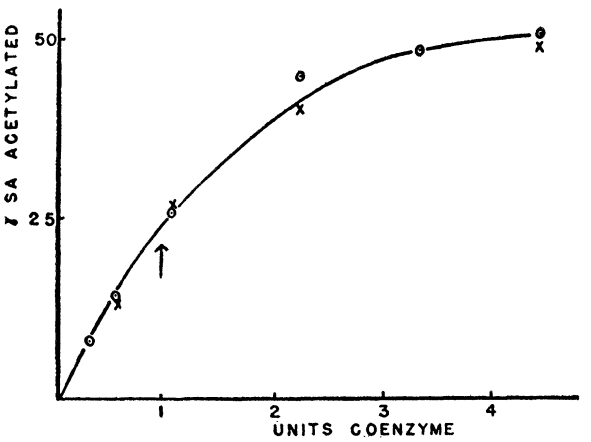


Fig. 4. Concentration-activity curves for coenzyme-A preparations of different purity. The arrow indicates the point of 1 unit on the curve. Ordinate scale, sulfanilamide acetylated. ○ represents crude coenzyme, 0.25 units/mg; x represents purified coenzyme, 130 units/mg.

were very fortunate to have the help of the great experience of Roger Williams' laboratory. Beverly Guirard, who occupied herself with this preparation, at first seemed not to find any appreciable amounts of the known vitamins. However, she became aware of the fact that on prolonged enzymatic treatment, the value of pantothenic acid, as determined microbiologically, did slightly increase. This gave the hint that the coenzyme may not release the pantothenic acid so easily, a fact well known from experience with pantothenic acid assay in tissue extracts. In confirmation, she found on acid hydrolysis of the coenzyme, considerable amounts of β-alanine, corresponding to 11 percent of pantothenic acid in this preparation which, as we now know, was 40-percent pure. The results of Guirard's vitamin survey, which gave us the practical assurance of the presence of pantothenic acid in the new coenzyme (*18*), are shown in Table 4.

The appearance of a B vitamin in the preparation was, of course, a most exciting event for our group and gave us further confidence that we were dealing here with a key substance. We still felt, however, slightly dissatisfied with the proof for pantothenic acid. Therefore, to liberate the chemically rather unstable pantothenic acid from CoA, we made use of observations on enzymatic cleavage of the coenzyme. Two enzyme preparations, intestinal phosphatase and an enzyme in pigeon liver extract, had caused independent inactivation. It then was found that through combined action of these two enzymes, pantothenic acid was liberated (*18, 19*).

Table 4. Vitamin content of preparation A.

Vitamin	Percentage
Nicotinic acid	0.06
Folic acid	.0002
Riboflavin	.006
Inositol	.05
Pyridoxine	.03
Biotin, thiamine	Not detectable
Pantothenic acid	
Direct	.085
1 wk incubated with papain-clarase	.16
From β-alanine, after acid hydrolysis	11.0

The two independent enzymatic cleavages indicated early that in CoA existed two independent sites of attachment to the pantothenic acid molecule. One of these obviously was a phosphate link, linking presumably to one of the hydroxyl groups in pantothenic acid. The other moiety attached to pantothenic acid, which cleaved off by liver enzyme, remained unidentified for a long time. In addition to pantothenic acid, our sample of 40-percent purity had been found to contain about 2 percent sulfur by elementary analysis and identified by cyanide-nitroprusside test as a potentential SH-grouping (*20, 21*). Furthermore, the coenzyme preparation contained large amounts of adenylic acid (*21*).

In the subsequent elaboration of the structure, the indications by enzyme analysis for the two sites of attachment to pantothenic acid have been most helpful. The phosphate link was soon identified as a pyrophosphate bridge (*22*). 5-Adenylic acid was identified by Novelli (*23*) as an enzymatic split product and by Baddiley (*24*), through chemical cleavage. At the same time, Novelli made observations that indicated the presence of a third phosphate in addition to the pyrophosphate bridge. These indications were confirmed by analysis of a nearly pure preparation that was obtained by Gregory (*25*) from *Streptomyces fradiae* in collaboration with the research group at the Upjohn Company (*26*). The generous help of the Upjohn Laboratories has been of great importance for the final identification of the structure of CoA. The analysis of this practically pure preparation is presented in Table 5.

It was at this period that we started to pay more and more attention to the sulfur in the coenzyme. As is shown in Table 5, our purest preparation contained 4.3 percent sulfur corresponding to 1 mole per mole of pantothenate. We also found (*26*) that dephosphorylation of CoA yielded a compound containing pantothenic acid and the sulfur-carrying moiety, which

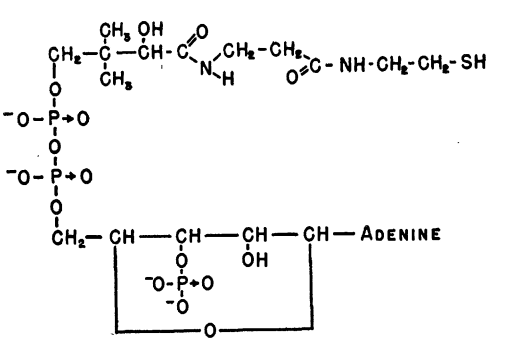


Fig. 5. Structure of coenzyme A.

Table 5. Composition of best preparation of CoA.

	Calcd. (%)*	Found (%)	Ratio
Pantothenic acid	28.6	26.8 (enzymatic assay) 25.6 (microbiological)	1
Adenine	17.6	17.0 (spectrophotometric)	1.05
Phosphorus (total)	12.12	10.6	2.83
Monoester phosphorus†		3.6	0.96
Sulfur	4.18	4.13	1.07

* Pantothenic acid, 2-mercaptoethylamine, 3 phosphoric acid, adenosine, $-5H_2O$; molecular weight 767.
† Liberated by prostate phosphomonoesterase.

we suspected as bound through the carboxyl. Through the work of Snell and his group (*27*), the sulfur-containing moiety proved to be attached to pantothenic acid through a link broken by our liver enzyme. It was identified as thioethanolamine by Snell and his group, linked peptidically to pantothenic acid.

Through analysis and synthesis, Baddiley now identified the point of attachment of the phosphate bridge to pantothenic acid in 4-position (*24*), and Novelli *et al.* (*28*) completed the structure analysis by enzymatic synthesis of "dephospho-CoA" from pantotheine-4′-phosphate and ATP. Furthermore, the attachment of the third phosphate was identified by Kaplan (*29*) to attach in 3-position on the ribose of the 5-adenlyic acid (whereas in triphosphopyridine nucleotide it happens to be in 2-position). Therefore, the structure was now established (Fig. 5).

Table 6. Coenzyme A in animal tissues. All values are given in units of coenzyme A per gram 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			

Table 7. Coenzyme A in microorganisms.

Microorganism	Coenzyme A per gram dry weight (units)	Preparation used for assay
Proteus morganii	572	Freshly grown organism, boiled
Lactobacillus arabinosus	150	
Lactobacillus delbrueckii	40	Dry preparation, suspended in water and boiled
Tray-dried yeast	72	
Tray-dried yeast	41	
Escherichia coli	320	
Propionic acid bacteria	330	
Clostridium butylicum (dried extract)	2000	

Metabolic Function of CoA

Parallel with this slow but steady elaboration of the structure, all the time we explored intensively metabolic mechanisms in the acetylation field. By use of the enzymatic assay, as is shown in Tables 6, 7, and 8, CoA was found present in all living cells, animals, plants, and microorganisms (*17*). Furthermore, the finding that all cellular pantothenic acid could be accounted for by CoA (*17*), made it clear that CoA represented the only functional form of this vitamin. The finding of the vitamin furnished great impetus; nevertheless, a temptation to connect the pantothenic acid with the acetyl transfer function blinded us for a long time to other possibilities.

The first attempts to explore further the function of CoA were made with pantothenic acid-deficient cells and tissues. A deficiency of pyruvate oxidation in pantothenic acid-deficient *Proteus morganii,* an early isolated observation by Dorfman (*30*) and Hills (*31*), now fitted rather well into the picture. We soon became quite interested in this effect, taking it as an indication for participation of CoA in citric acid synthesis. A parallel between CoA levels and pyruvate oxidation in *P. morganii* was demonstrated (*32*). Using pantothenic acid-deficient yeast, Novelli *et al.* (*33*) demonstrated a CoA-dependence of acetate oxidation (Fig. 6) and Olson and Kaplan (*34*) found with duck liver a striking parallel between CoA content and pyruvic utilization, which is shown in Fig. 7.

But more important information was being gathered on the enzymatic level. The first example of a generality of function was obtained by comparing the acti-

Table 8. Coenzyme A in plant material.

	Coenzyme A per gram fresh weight (units)
Spinach	0.74
Tomato	1.3
Frozen peas	4.5
Wheat germ (commercial sample)	30
Royal jelly (bee)	0

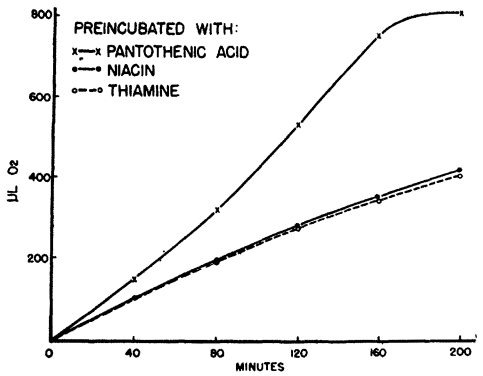


Fig. 6. Effect of coenzyme A on acetate oxidation in yeast.

vation of apoenzymes for choline and sulfonamide acetylation, respectively, using our highly purified preparations of CoA (*9*). As is shown in Fig. 8, similar activation curves obtained for the two respective enzymes. Through these experiments, the heat-stable factor for choline acetylation that had been found by Nachmansohn and Behrman (*35*) and by Feldberg and Mann (*36*) was identified with CoA.

The next most significant step toward a generalization of CoA function for acetyl transfer was made by demonstrating its functioning in the enzymatic synthesis of acetoacetate. The CoA effect in acetoacetate

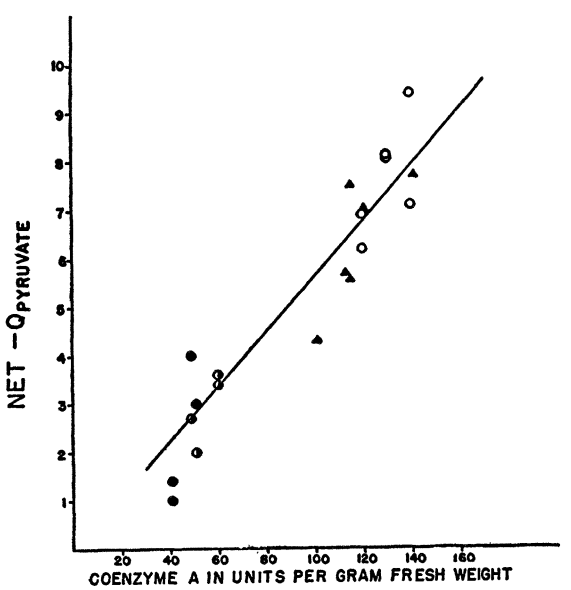


Fig. 7. Relationship between net pyruvate utilization (net-$Q_{pyruvate}$) and coenzyme-A content of liver slices from deficient, pantothenic acid-treated, and normal ducks. Net-$Q_{pyruvate}$ values for individual liver slices are plotted against their respective coenzyme-A values in units per gram of fresh weight of slice. ● represents deficient; ◑, deficient treated *in vitro*; ▲ deficient treated *in vivo* by intraperitoneal injection of 10 mg of calcium pantothenate per 100 g of body weight 1 to 2 hr before observation; ○ normal controls fed *ad libitum*.

synthesis was studied by Morris Soodak (*37*), who obtained for this reaction a reactivation curve quite similar to the curves for enzymatic acetylation (Fig. *9*). Soon afterwards Stern and Ochoa (*38*) showed a CoA-dependent citrate synthesis with a pigeon liver fraction similar to the one used by Soodak for acetoacetate synthesis. In our laboratory, Novelli *et al.* confirmed and extended this observation with extracts of *Escherichia coli* (*39*).

In the course of this work, which more and more clearly defined the acetyl transfer function of CoA, Novelli once more tried acetyl phosphate. To our surprise and satisfaction, it then appeared, as is shown in Table 9, that in *E. coli* extracts, in contrast to the animal tissue, acetyl phosphate as acetyl donor for citrate synthesis was more than twice as active as ATP-acetate (*39*). Acetyl phosphate, therefore, functioned as a potent microbial acetyl donor. Acetyl transfer from acetyl phosphate, like that from ATP-acetate, was CoA-dependent, as is shown in Table 9. Furthermore, a small amount of "microbial conversion factor," as we called it first, primed acetyl phosphate for activity with pigeon liver acetylation systems, as is shown

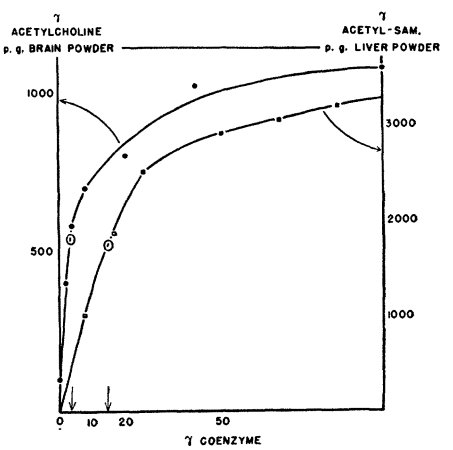


Fig 8. Activation curves for acetocholinekinase and acetoarylaminekinase by purified coenzyme-A preparations.

Table 9. Citric acid synthesis in dialyzed extract of *E. coli*. All tubes contained 1.0 ml of extract, 0.025*M* oxalacetic acid, 0.016*M* $NaHCO_3$, 0.02*M* $MgCl_2$, and 0.01*M* cysteine in a final volume of 2.5 ml. The concentrations of the additions were as follows: sodium acetate 0.05*M*, sodium ATP 0.02*M*, lithium acetyl phosphate 0.004*M*, and coenzyme A 17 units.

Additions	Citric acid synthesized per milliliter extract (μM)
None	0
Acetate, ATP	0.23
Acetate, ATP coenzyme A	1.30
Acetyl phosphate	0.25
Acetyl phosphate coenzyme A	4.0

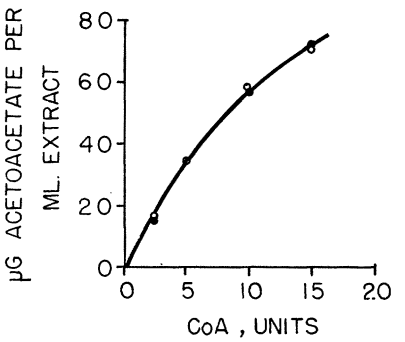


Fig. 9. Coenzyme-A-dependence of acetoacetate formation from acetate plus ATP with ammonium sulfate pigeon liver fraction.

in Table 10 (*40*). Eventually the microbial conversion factor was identified by Stadtman *et al.* (*40*) with the transacetylase first encountered by Stadtman and Barker in extracts of *Clostridium kluyveri* (*41*) and likewise, although not clearly defined as such, in extracts of *E. coli* and *C. butylicum* by Lipmann and Tuttle (*42*).

The definition of such a function was based on the work of Doudoroff *et al.* (*43*) on transglucosidation with sucrose phosphorylase. Their imaginative use of isotope exchange for closer definition of enzyme mechanisms has been most influential. Like glucose-1-phosphate with sucrose phosphorylase, acetyl phosphate with these various microbial preparations equilibrates its phosphate rapidly with the inorganic phosphate of the solution. As in the Doudoroff *et al.* experiments, first a covalent substrate enzyme derivative had been proposed (*43*). However, then Stadtman *et al.* (*40*), with the new experience of CoA-dependent acetyl transfer, could implicate CoA in this equilibration between acetyl- and inorganic phosphate and thus could define the transacetylase as an enzyme equilibrating acetyl between phosphate and CoA:

$$\text{Acetyl} \sim \text{OPO}_3^{=} + \text{CoASH} \xleftrightarrow{\text{transacetylase}} \text{acetyl} \sim \text{SCoA} + \text{HOPO}_3^{=}$$

Table 10. Acetylation of p-aminobenzoic acid (PABA) by pigeon liver enzyme, acetyl phosphate, and *C. kluyveri* extract. Conditions, tris(hydroxymethyl)aminomethane buffer (*p*H 8.1), 0.2*M*; cysteine, 0.01*M*; acetyl ~ P, 0.025*M*; PABA, 0.001*M*; CoA (67 units per milligram), 10 units; bacterial transacetylase (acid ammonium sulfate fraction, 43 to 86 percent saturation), 0.3 mg; pigeon liver fraction (A-60-4), 0.3 ml. Final volume 1.0 ml, 28°, 60 min.

System	Acetyl PABA, Bratton and Marshall (μM)
Acetyl ~ P + liver fraction, A-60	0
Acetyl ~ P + transacetylase	0
Transacetylase + liver fraction	0
Acetyl ~ P + liver fraction + bacterial transacetylase	0.92

In the course of these various observations, it became quite clear that there existed in cellular metabolism an acetyl distribution system centering around CoA as the acetyl carrier that was rather similar to the ATP-centered phosphoryl distribution system. The general pattern of group transfer became recognizable, with donor and acceptor enzymes being connected through the CoA ⟷ acetyl CoA shuttle.

A clearer definition of the donor-acceptor enzyme scheme was obtained through acetone fractionation of our standard system for acetylation of sulfonamide into two separate enzyme fractions, which were inactive separately but showed the acetylation effect when combined. A fraction, A-40, separating out with 40 percent acetone, was shown by Chou (*44*) to contain the donor enzyme responsible for the ATP-CoA-acetate reaction, whereas with more acetone, the acceptor function, A-60, precipitated the acetoarylaminekinase, as we propose to call this type of enzyme. The need for a combination of the two for over-all acetyl transfer is shown in Fig. 10. This showed that a separate system was responsible for acetyl CoA formation through interaction of ATP, CoA, and acetate (see later) and that the over-all acetylation was a two-step reaction:

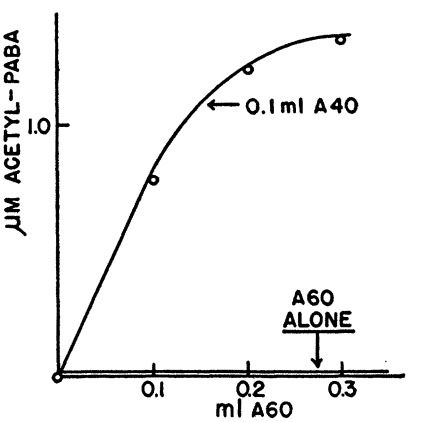


Fig. 10. Acetylation of p-aminobenzoic acid (PABA) with fractions A-40 and A-60 added in various proportions.

$$\text{ATP} + \text{CoA} + \text{acetate} \rightleftharpoons \text{acetyl CoA} + \text{AMP} + \text{pyrophosphate} \quad (1)$$

$$\text{Acetyl CoA} + \text{arylamine} \rightarrow \text{acetyl arylamine} + \text{CoA} \quad (2)$$

These observations crystallized into the definition of a metabolic acetyl transfer territory as pictured in Fig. 11.

This picture had developed from the growing understanding of enzymatic interplay involving metabolic generation of acyl CoA and transfer of the active acyl to various acceptor systems. A most important, then still missing link in the picture was supplied through the brilliant work of Feodor Lynen (*45*) who chemically identified acetyl CoA as the thioester of CoA. Therewith the thioester link was introduced as a new energy-rich bond, and this discovery added a very novel facet to our understanding of the mechanisms of metabolic energy transformation.

Carboxyl and Methyl Activation in Acetyl CoA

In spite of many similarities between the general aspects of group transfer involving phosphoryl and acetyl groupings, there is a considerable difference insofar as the grouping transferred in the acetyl territory is an organic grouping and displays a quite different versatility for condensation reactions, yielding eventually large and complex carbon structures. There is one feature in this picture that always has attracted our particular attention: the twofold type of activation involving (i) the carboxyl end, or the "head," of the acetyl and (ii) its methyl, or "tail," end.

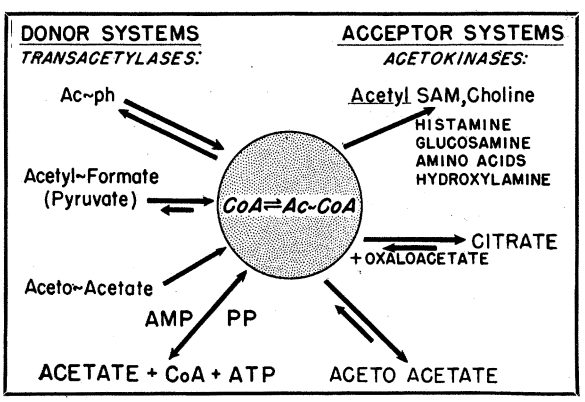


Fig. 11. Acetyl transfer scheme.

The definition of the head reaction is relatively simple. Acetylation of arylamine or choline is a typical head reaction. There is to be mentioned, furthermore, the observation by Chantrenne (*46*), introducing CoA as a rather general catalyst of acyl activation. He demonstrated the activity of CoA in benzoyl transfer such as hippuric acid synthesis. The mechanism of this synthesis was elaborated recently by Taggart (*47*), who clearly defined benzoyl CoA as the benzoyl donor in this reaction. An even greater and more prominent generalization is offered through the more and more developing importance of succinyl CoA in intermediary metabolism.

The second type, the methyl, or tail, activation, is

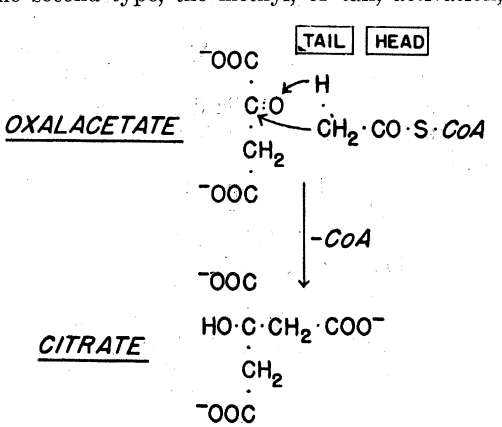


Fig. 12. Tail reaction: citric acid synthesis.

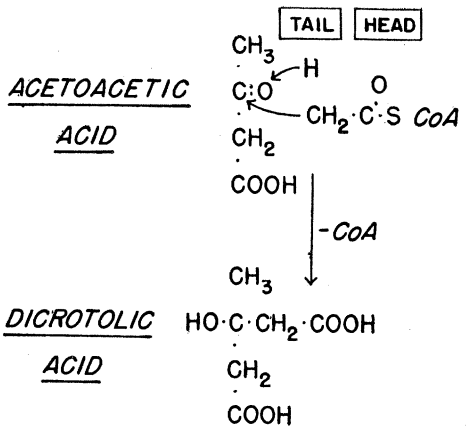


Fig. 13. Tail reaction: synthesis of β-hydroxy, β-methyl glutaric acid (dicrotolic acid). Note the similarity to citric acid synthesis.

not as well understood. In citric acid synthesis, as may be seen from Fig. 12, the methyl end engages in an aldolase type of condensation with the carbonyl group of the oxalacetate as acceptor. This condensation requires an energy input that must be derived from the thioester link, and at the end of the reaction CoA appears to be liberated in some manner.

The complexity of the citrate condensation is emphasized through the existence of an ATP-CoA-citrate reaction recently observed by Srere *et al.* (*48*), which results in the disruption of citrate to oxalacetate and acetyl CoA.

$$\text{CoA} + \text{ATP} + \text{citrate} \rightleftharpoons \text{acetyl} \cdot \text{CoA} + \text{oxalacetate} + \text{ADP} + \text{P}$$

The mechanism remains still to be understood in greater detail. The reaction is mentioned here because it introduced the new variety into the citric acid cycle through a conversion of phosphoryl via citrate into acetyl.

For a long time the citrate reaction was the only known tail condensation. However, recently another interesting example has developed in the study of the precursors in steroid and isoprene synthesis. The initial condensation product in this series appears to be β-methyl, β-hydroxyglutarate (dicrotolic acid), formed through condensation of acetoacetate with acetyl CoA. The striking analogy between this and the citrate condensation appears in Fig. 13. This initial condensation seems to be followed by decarboxylation and dehydration to β-methyl crotonic acid first demonstrated by Bonner *et al.* (*49*) as intermediary in rubber synthesis.

In a third type, a combination of head *and* tail reaction takes place with two acyl CoA's reacting with each other in a head-to-tail condensation. When studying acetoacetate synthesis, we were at first not quite aware of its belonging to this third type of reaction. The calculation of the energy required had yielded a figure of around 15 kilocalories which could be covered by one energy-rich bond (*9*). However, by using as

Table 11. Synthesis of acetoacetate from $CH_3C^{14}OOPO_3^=$

	Start	End
	(counts per min, per μM)	
Acetate	0	2,900
Acetyl phosphate	23,000	22,400
Acetoacetate		35,200
Carboxyl-C		15,700
Carbonyl-C		19,500

acetyl donor carboxyl-labeled acetyl phosphate, fed through transacetylase, the marker appeared in the carbonyl, as well as the carboxyl, part of acetoacetate (Table 11). This demonstrated a head-tail character for the reaction. The finer mechanism of this reaction between two acetyl CoA's, as shown in Fig. 14, has

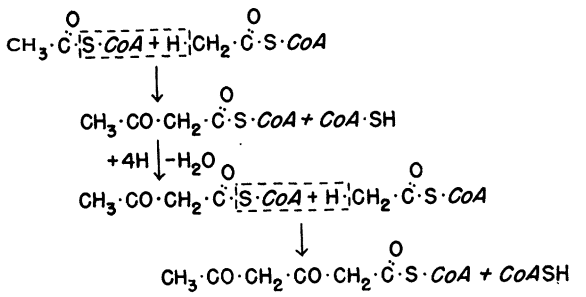


Fig. 14. Head-to-tail condensation: acetoacetate synthesis and follow reactions in straight-chain fatty acid synthesis.

been more recently elaborated in particular by Lynen's group (*50*), by Green's laboratory (*51*) and by Ochoa and his group (*52*). Presumably in the building up of longer terpene chains, a head-tail condensation may occur between two β-methyl crotonyl CoA's, followed by hydrogenation and dehydration (Fig. 15).

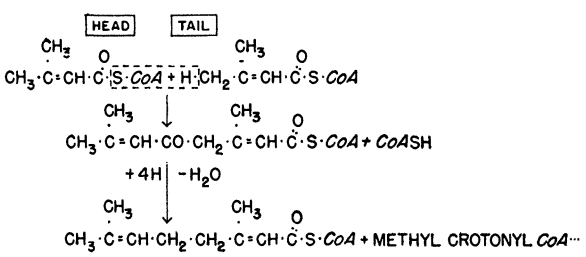
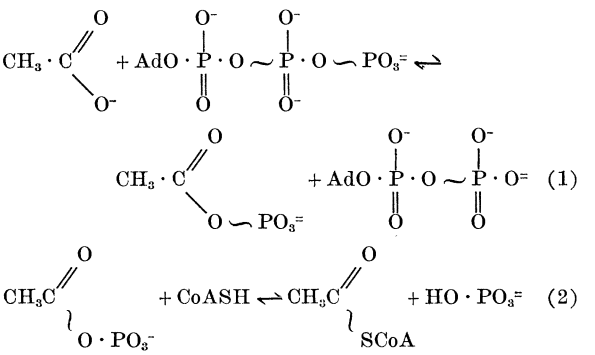


Fig. 15. Tentative scheme for terpene synthesis: head-to-tail condensation of methyl-crotonyl CoA's.

A quite new type of head-tail condensation, presumably between succinyl CoA and glycyl CoA was recently suggested by Shemin's work on hemin synthesis (*53*). The thus primarily formed keto, amino dicarboxylic acid then appears to be decarboxylated to δ-amino levulinic acid. Shemin synthesized the latter marked with C^{14} and showed its incorporation into the heme molecule.

Mechanism of ATP-CoA-Acetate Reaction

In the recent past we have been mostly occupied with the mechanism through which the phosphate bond in ATP converts to acetyl bond in acetyl CoA. In animal tissue where acetyl phosphate appeared not to be an intermediate, the conversion mechanism remained very puzzling for a long time. Before considering this reaction, it will be advantageous to review first the microbiological mechanism of such interconversion and in particular the role of acetyl phosphate as an intermediary. This transformation is rather straightforward: a sequence of two independent enzymatic reactions, the first a transphosphorylation from ATP to acetate and the second, as discussed, a transacylation from acetyl phosphate to CoA:



It should be noted that in the first transphosphorylation step the acetyl phosphate cleaves and condenses between O and P. In the second transacylation reaction, however, acetyl phosphate cleaves and condenses between carbon C and O. Thus the same molecule reacts on each side of the oxygen bridge between the carbon and the phosphorus. This shift of the site of cleavage in the sequence is significant. This possibility attracted my early attention (see Fig. 3) and was one of the reasons that prompted me into this whole exploration. A shift from P—OC to PO—C should actually be a feature of many condensations initiated by a phosphoryl split from ATP. These become increasingly numerous, such as in glutamine, glutathione, pantothenate, and seemingly in protein synthesis.

The finer mechanism generally is obscured by enzyme-bound steps. In all these reactions, however, somewhere along the line a shift from transphosphorylation to transacylation sems to be inherent. This shift stands out very clearly in the microbial two-step reaction. But in animal tissue, the energy transmission from phosphoryl to acetyl occurs through a continuous enzyme-bound reaction chain which is more difficult to elucidate. Nevertheless, some progress has been made, which also starts to reflect on other mechanisms of this type.

Jones *et al.* (*54, 55, 56*) have explored the reaction with liver and with yeast, and a surprising feature was uncovered, namely, that the initial phosphoryl split of ATP occurs between pyrophosphoryl group and AMP. The cleavage products of ATP were identified as inorganic pyrophosphate (PP) and adenylic

Table 12. Effect of fluoride on pyrophosphate formation. Each vessel contained in 1 ml: 12 μM ATP; 10 μM potassium acetate; 10 μM $MgCl_2$; 20 μM H_2S; 200 μM tris(hydroxymethyl)aminomethane buffer, *p*H 7.5 and 0.02 ml (20 units) of yeast enzyme fraction 4. Vessels were incubated at 37°C for 30 min.

Fluoride added (μM/ml)	CoA* added (μM/ml)	Acetyl CoA (μM/ml)	PP (μM/ml)	Pi (μM/ml)
0	0	0	0	7.4
0	2.9	2.72	0	13.2
50	0	0	0	1.75
50	2.9	2.88	3.10† 3.16‡	2.10

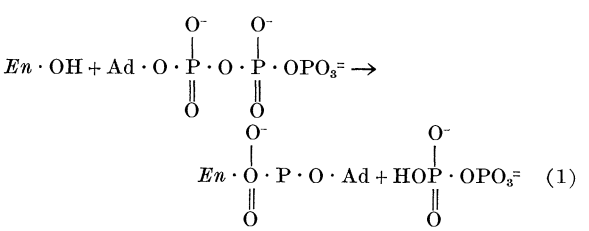
* 1 μM CoA = 310 units.
† Determined by color increase.
‡ Determined by Mn precipitation method.

acid (AMP). The mechanism was obscured by the presence of pyrophosphatase which, however, could be suppressed with fluoride. Table 12 shows the course of reaction in the presence and the absence of fluoride, using the hydroxamate formation as an index.

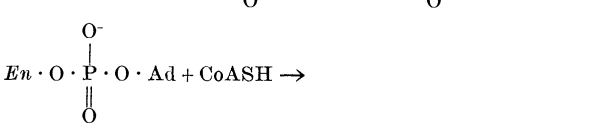
Some further rather revealing information was obtained by the use of isotopes. This was suggested by Lynen during his visit to our laboratory (*50*). It was found that ATP and radioactive inorganic pyrophosphate exchange in the absence of CoA or acetate. Such an exchange is best compatible with an initial reaction between the enzyme and ATP, resulting in a covalent binding of AMP to the enzyme, E,

$$ATP + E \rightleftharpoons AMP—E + PP$$

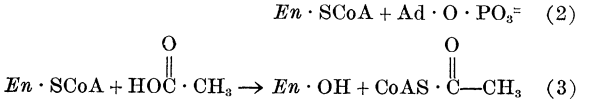
It furthermore was found that acetyl CoA exchanges with radioactive free acetate in the absence of ATP or pyrophosphate. This exchange would indicate an exchange of acetyl for enzyme in the final step. Therefore, an over-all sequence (*En* standing for enzyme) was proposed as follows:



(1)



(2)



(3)

The middle step—that is, the substitution of enzyme-bound AMP by CoA—is the most problematic but also the most interesting one, since it may foreshadow mechanisms implicating nucleotide activation for polynucleotide formation. No indications were found for the identity of the grouping on the enzyme through which the initial binding of AMP and further exchange with CoA might occur. A further purification of the enzyme would be necessary before obtaining fuller information.

A pyrophosphoryl split of ATP also was found by Maas (*57, 58*) to initiate pantothenate synthesis. The isotope exchange picture with this synthesis is somewhat different. It indicates in some manner a phosphorylative priming for peptidic synthesis and has been very tentatively used as a start for developing possible schemes for polypeptide synthesis (*59*).

Altogether, in this area, a diverstified picture is rapidly developing. There is good reason to hope that in the not too distant future, out of the fair confusion of the present, a clearer understanding will eventually evolve. A new level of complexity seems slowly to unravel, and the gap between the biochemical and biological approach is narrowing down.

References

1. F. Lipmann, *Cold Spring Harbor Symposia Quant. Biol.* **7**, 248 (1939).
2. Kämmerer and Carius, *Ann.* **131**, 165 (1864).
3. F. Lipmann, *J. Biol. Chem.* **134**, 463 (1940).
4. ———, *ibid.* **155**, 55 (1944).
5. ———, and L. C. Tuttle, *ibid.* **153**, 571 (1944).
6. ———, *ibid.* **159**, 21 (1945).
7. F. Lipmann, *Advances in Enzymol.* **1**, 99 (1941).

7a. R. Bentley, *Cold Spring Harbor Symposia Quant. Biol.* **8**, 11 (1948).

8. F. Lipmann, in *Currents in Biochemical Research*, D. E. Green (Ed.) (Interscience, New York, 1946), p. 137.
9. F. Lipmann, *Advances in Enzymol.* **6**, 231 (1946).
10. E. R. Stadtman and F. Lipmann, *J. Biol. Chem.* **185**, 549 (1950).
11. F. Lipmann, *ibid.* **160**, 173 (1945).
12. W. O. Sykes, *Biochem. J.* **38**, XXIX (1944).
13. J. R. Klein and J. S. Harris, *J. Biol. Chem.* **124**, 613 (1938).
14. H. Kalckar, *Biochem. J.* **33**, 631 (1939).
15. D. Nachmansohn, H. M. John, and H. Waelsch, *J. Biol. Chem.* **150**, 485 (1943).
16. D. Nachmansohn and A. L. Machado, *J. Neurophysiol.* **6**, 397 (1943).
17. N. O. Kaplan and F. Lipmann, *J. Biol. Chem.* **174**, 37 (1948).
18. F. Lipmann *et al., ibid.* **167**, 869 (1947).
19. G. D. Novelli, N. O. Kaplan, and F. Lipmann, *ibid.* **177**, 97 (1949).
20. F. Lipmann, N. O. Kaplan, and G. D. Novelli, *Federation Proc.* **6**, 272 (1947).
21. F. Lipmann *et al., J. Biol. Chem.* **186**, 235 (1950).
22. G. D. Novelli, N. O. Kaplan, and F. Lipmann, *Federation Proc.* **9**, 209 (1950).
23. G. D. Novelli, *Physiol. Revs.* **33**, 525 (1953).
24. J. Baddiley and E. M. Thain, *J. Chem. Soc.* (Sept., 1951), p. 2253.
25. J. D. Gregory, G. D. Novelli, and F. Lipmann, *J. Am. Chem. Soc.* **74**, 854 (1952).
26. W. H. DeVries *et al., ibid.* **72**, 4838 (1950).
27. E. E. Snell *et al., ibid.* **72**, 5349 (1950).
28. J. Baddiley *et al., Nature* **171**, 76 (1953).
29. L. S. Shuster and N. O. Kaplan, *J. Biol. Chem.* **201**, 535 (1953).
30. A. Dorfman, S. Berkman, and S. A. Koser, *ibid.* **144**, 393 (1942).
31. G. M. Hills, *Biochem. J.* **37**, 418 (1943).
32. G. D. Novelli and F. Lipmann, *Arch. Biochem.* **14**, 23 (1947).
33. G. D. Novelli and F. Lipmann, *J. Biol. Chem.* **171**, 833 (1948).
34. R. E. Olson and N. O. Kaplan, *ibid.* **175**, 515 (1948).
35. D. Nachmansohn and M. Berman, *ibid.* **165**, 551 (1946).
36. W. Feldberg and T. Mann, *J. Physiol.* **104**, 411 (1946).
37. M. Soodak and F. Lipmann, *J. Biol. Chem.* **175**, 999 (1948).
38. T. R. Stern and S. Ochoa, *ibid.* **179**, 491 (1949).

39. G. D. Novelli and F. Lipmann, *ibid.* **182**, 213 (1950).
40. E. R. Stadtman, G. D. Novelli, and F. Lipmann, *ibid.* **191**, 365 (1951).
41. E. R. Stadtman and H. A. Barker, *ibid.* **184**, 769 (1950).
42. F. Lipmann and L. C. Tuttle, *ibid.* **158**, 505 (1945).
43. M. Doudoroff, H. A. Barker, and W. Z. Hassid, *ibid.* **168**, 725 (1947).
44. T. C. Chou and F. Lipmann, *ibid.* **196**, 89 (1952).
45. F. Lynen, E. Reichert, and L. Rueff, *Ann.* **574**, 1 (1951).
46. H. Chantrenne, *J. Biol. Chem.* **189**, 227 (1951).
47. D. Schachter and J. V. Taggart, *J. Biol. Chem.* **203**, 925 (1953).
48. P. A. Srere and F. Lipmann, *J. Am. Chem. Soc.* **74**, 4874 (1953).
49. J. Bonner and B. Arreguin, *Arch. Biochem.* **21**, 109 (1949).
50. F. Lynen, *Harvey Lectures* **48**, 212 (1954).
51. D. E. Green, S. Mii, and H. R. Mahler, *J. Biol. Chem.* **206**, 1 (1954).
52. S. Ochoa, *Advances in Enzymol.* **15**, 183 (1954).
53. D. Shemin and C. S. Russel, *J. Am. Chem. Soc.* **75**, 4873 (1953).
54. M. E. Jones *et al.*, *ibid* **75**, 3285 (1953).
55. M. E. Jones *et al.*, *Biochem. et Biophys. Acta* **12**, 141 (1953).
56. M. E. Jones, *Federation Proc.* **12**, 708 (1953).
57. W. K. Maas, *ibid.* **12**, 241 (1953).
58. W. K. Maas and G. D. Novelli, *Arch. Biochem. Biophys.* **43**, 236 (1953).
59. F. Lipmann, in *Mechanism of Enzyme Action*, W. D. McElroy and B. Glass, Eds. (Johns Hopkins Press, Baltimore, 1954), p. 599.

This morning, looking out of our window, I was deeply moved to see that the flags had come out to honor men whose only claim to fame may seem that they succeeded in finding answers to some of the mysteries of nature. Their purpose often may be none but just to push back a little the limits of our comprehension. Their findings mostly have to be expressed in a scientific language that is understood by only a few. We feel, nevertheless, that the drive and urge to explore nature in all its facets is one of the most important functions of humanity. To make the general public truly aware of such seems to me one of the great achievements of the Nobel Institution.—[Remarks by Fritz Lipmann at the Nobel banquet, 10 Dec. 1953. See page 855.]

International Conference on Nuclear Physics in Glasgow

Rolf M. Steffen

Department of Physics, Purdue University, Lafayette, Indiana

ABOUT 300 physicists representing some 20 countries attended an international conference on nuclear physics held at the University of Glasgow 12–17 July. Sponsored by the International Union of Pure and Applied Physics and UNESCO, the conference was magnificently organized by the staff of the Glasgow Physics Department headed by P. I. Dee.

During the first 2 days of the conference attention was focused on problems of nuclear forces and nuclear scattering as well as on discussions of nuclear spectroscopy and on the understanding of nuclear data in view of the different nuclear models. After ample time for informal discussions provided by a river cruise on the third day, the conference continued with a somewhat more diversified program for the remaining days. The topics discussed included beta- and gamma-ray transitions, photodisintegration, theory of nuclear-energy levels, field theory, meson theory in general, π-mesons, high-energy experiments, and the production of heavy mesons and hyperons.

Neither spectacular progresses in theory nor excitingly new experimental results were reported in the 105 papers that were presented, but the conference offered an excellent opportunity for reviewing and summarizing the present position of nuclear and meson theory in view of the recently accumulated experimental data. Considering the wealth of material presented, only a limited number of papers can be discussed here in detail. A comprehensive report of the conference will be found in *Proceedings of the International Conference on Nuclear Physics, Glasgow 1954* (Pergamon Press, London), in press.

Following the welcome address by Dee, the first session, which was devoted entirely to the subject of *nuclear forces and nuclear scattering,* was opened by H. S. W. Massey (University College, London) with a review of the present status of the empirical study of nuclear forces. The information on these forces extracted from *low-energy* (up to 10 Mev) two-body phenomena such as proton-proton (p-p) and neutron-proton (n-p) scattering seems to be consistent with the charge-independence hypothesis of nuclear forces. The experimentally observed difference in the singlet scattering lengths of the p-p scattering and the n-p scattering can be accounted for by electromagnetic effects. Concerning the noncentral aspects of the nuclear forces, characterized by the tensor compo-