single or few gene differences or simple structural heterozygosity leads to the formation of nonviable combinations. Incipient species may owe their origins to differences such as these, but the accumulation of further differences must follow before what was once a single species may be considered as two. It is probably undesirable that there be any agreement concerning the exact degree of introgression which might be allowed between two taxa which yet remain as separate species. When, however, the differences between the two taxa become appreciably greater than the variation within each of these taxa, the two are certainly approaching the level of species. Conversely, the occurrence of as great or greater variation within two taxa than between them must place the two taxa in subspecific rank under a single species. The latter point of view will be used to show that two long-recognized species of *Oenothera* are probably only variations of a single species.

In a general survey of the cytogenetics of *Oenothera* subgenus *Raimannia,* Hecht (*1*) pointed out that *Oenothera affinis* Camb. and *Oenothera mollissima* L. might represent extremes in variation of a single species. This possibility has been considerably strengthened following a more detailed study involving six races of each of these two species (*2*). Morphologically *O. affinis* is distinguished from *O. mollissima* by its longer hypanthia and longer petals; cytologically, it was found that all the races of *O. mollissima* are complex heterozygotes with a circle of 14 chromosomes, whereas *O. affinis* includes complete homozygotes, intermediate types, and complex heterozygotes like those of *mollissima.*

Specific relationships in *Oenothera* are complicated by the fact that circle-of-14 types are a special type of species, consisting of two distantly related genomes maintained in a condition of permanent heterozygosity by virtue of a system of balanced lethals which prevents the formation or survival of the homozygous combinations. However, when two circle-of-14 races are crossed, the progeny may show pairing of at least some of their chromosomes. The number of pairs thus obtained is a measure of the relatively recent common origin of the chromosomes of the two genomes combined in the new hybrid, and therefore is a measure of the relationship of the parental genomes.

Six geographical races each of *O. affinis* and *O. mollissima* were crossed with each other in all possible combinations. Mature progeny were obtained from 28 (30 possible) of each of the intraspecific crosses and from 63 (72 possible) of the interspecific crosses. Many of these hybrids included two to four classes of progeny which differed in chromosome configuration, and may therefore be considered as different combinations of genomes. Combinations that indicated a difference of no more than 3 interchanges were considered to indicate a relatively close relationship, whereas 4 to 6 interchanges were considered as indicative of a more remote common origin of the genomes involved. Of the 50 interracial *affinis* combinations 39 (78%) differed by 3 or less than 3 interchanges. Only 4 of the 43 interracial *mollissima* combinations (10%) differed by 3 or fewer interchanges. Thirty-one per cent (34 out of a total of 108) of the interspecific combinations were similarly found to have closely related genomes. It appears, thus, that the genomes of *affinis* are more closely related to those of *mollissima* than are the *mollissima* complexes to each other. *O. affinis* and *O. mollissima* have essentially contiguous distributions in southern South America (*3*), and the evidence above strongly suggests that introgression occurs. Under these circumstances it is probable that *O. affinis* Camb. (and its several synonyms) should be included under the prior epithet *O. mollissima* L.

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Manuscript received August 14, 1953.

Protein Metabolism and Interactions[1]

Robert L. Evans and Donald S. Amatuzio[2, 3]

Department of Mechanics and Materials, University of Minnesota, and the Department of Medicine, Veterans Administration Hospital, Minneapolis, Minnesota

This note applies and extends the methods of Sprinson and Rittenberg for the interpretation of protein metabolism and interactions (*1*). There is now available a sufficient quantity of experimental results (from widely scattered sources) to form the basis of an outline of body protein reactions.

The main or overall protein reactions may be interpreted on the basis of the simplified scheme of Fig. 1. The k's represent rate constants, and all the reaction rates are assumed to be first order with respect to the reactant.

Let A be the total amount of body protein and let f_j ($j=1$, 2, 3, or 4) be the fraction of that which is in protein x_j. Then if x_{js} is the amount of protein x_j when a balanced steady state is maintained, it follows that $x_{js}=f_jA$. In this formulation k_{j5} is the turnover rate of protein x_j, and the fraction $k_{05}/(k_{01}+k_{02}+\cdots+k_{05})$ may be called the fraction of exogenous protein. This fraction, $k_{05}/(k_{01}+\cdots+k_{05})$, is given by the A of Sprinson and Rittenberg's Eq. (1) (*1*). They found that this fraction is about 0.5 in normal adults on diets of their own choice. They also found that the corresponding substance, y, has a half-time of only about one-half day in the body. The results of Maas *et al.* (*2*) on the injection of rats with radioactive

[1] Sponsored by the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors do not necessarily reflect the opinion and policy of the Veterans Administration.

[2] The authors thank Dr. H. S. Wells, Director of Research at the Veterans Administration Hospital, for encouragement and for providing the atmosphere of free inquiry that is essential to research.

[3] Dr. Amatuzio's present address: U.S. Naval Hospital, Oakland, Calif.

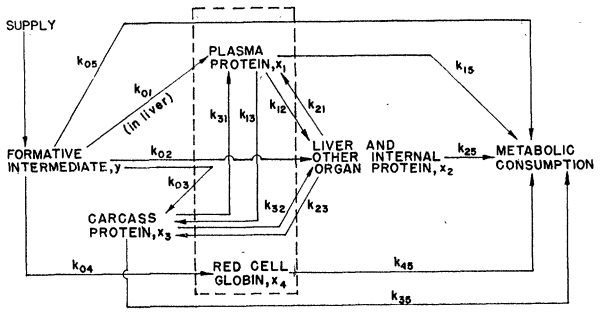

FIG. 1. Simplified scheme of the main or overall protein reactions. The dotted box encloses portions of the scheme involving blood components or transportation by blood.

sulfur show tagging peaks of short duration in the liver and kidney. These peaks probably represent some of the exogenous fraction of y. The individuals on the minimum essential diets of Rose (*3*) were probably getting negligible quantities of protein that would enter this exogenous fraction of y. In other words, k_{05} was then approximately zero.

When N^{15}-tagged amino acid is fed over a period of a few days, most of the body protein tagging is that of x_1, x_2, x_3, and x_4, which may be called endogenous protein (*4, 5*). Let

$$T_j = 1/k_{j5} \tag{1}$$

be called the average utilization time of protein x_j. This is observed by the isotopic tagging of one constituent element, such as nitrogen, so it refers to the utilization of that element in the protein x_j ($j = 1, 2, 3,$ or 4). Let

$$H_j = (\text{a constant}) \cdot k_{0j}/f_j \tag{2}$$

be the greatest initial tagging, or curve height, for protein x_j (often in units of atom per cent excess). The constant in Eq. (2) is independent of j and is a true constant for any individual in a balanced steady state. To maintain such balance

$$f_1k_{15}/k_{01} = f_2k_{25}/k_{02} = f_3k_{35}/k_{03} = f_4k_{45}/k_{04} \tag{3}$$

From Eqs. (1), (2), and (3)

$$H_1T_1 = H_2T_2 = H_3T_3 = H_4T_4 \tag{4}$$

As far as globin is concerned, the above equations are equivalent representations that accurately portray only the average values. In actuality, globin is incorporated in red cells by the bone marrow, these are gradually released to the circulating blood, and the globin is essentially inert until its red cells break down several months later. The experimental studies of tagged globin (*6, 7*) may be interpreted as showing that T_4 is about 130 days (*8*). Sprinson has stated (*9*) that in the experiment of our reference (*7*) the observed H_1/H_4 was about 4.6, so from Eq. (4) T_1 is about 28 days.

Also let T_t be the average overall utilization time for the whole body so that

$$f_1/T_1 + f_2/T_2 + f_3/T_3 + f_4/T_4 = 1/T_t \tag{5}$$

Since the average 40-kg man has a little over 4 kg of protein, Rose's findings (*3*) imply that T_t is about 100 days. The experiments of Shemin and Rittenberg on rats (*4*) show that $H_2/H_3 = T_3/T_2$ is about 5. These values, together with reasonable estimates of the f_j's, convert Eq. (5) to

$$0.05/28 + 0.18/T_2 + 0.65/5T_2 + 0.12/130 = 1/100$$

Thus T_2 is about 42 days and $T_3 = 5T_2$ is about 210 days. A summary of these overall average values is:

Protein	x_1	x_2	x_3	x_4	Total
Fraction of total, f_j	0.05	0.18	0.65	0.12	1.00
Av. util. time, T_j (days)	28	42	210	130	100
$k_{j5} = 1/T_j$	0.036	0.024	0.0048	0.0076	0.010

This formulation implies that the average isotopic excess of the whole body decreases daily about 1/100th of its value throughout the course of tagging experiments.

All body proteins except globin appear also to be involved in active interactions, because all their isotope curves eventually approach a level of slow decline at or near that of the average isotope concentration. According to the scheme suggested here, these interaction reactions would satisfy the differential system.

$$\begin{aligned}
\frac{dx_1}{dt} &= k_{21}x_2 + k_{31}x_3 - (k_{12} + k_{13} + k_{15})x_1 \\
\frac{dx_2}{dt} &= k_{12}x_1 + k_{32}x_3 - (k_{21} + k_{23} + k_{25})x_2 \\
\frac{dx_3}{dt} &= k_{13}x_1 + k_{23}x_2 - (k_{31} + k_{32} + k_{35})x_3
\end{aligned} \tag{6}$$

$x_j = x_j(t)$ = amount of isotopic excess in protein x_j at time t (const. $\times f_j \times$ at % excess), and

$x_j(0) = H_j \quad (j = 1, 2, \text{ or } 3)$

The solution of this system has the form

$$x_j = C_{j1} \exp(-r_1t) + C_{j2} \exp(-r_2t) + C_{j3} \exp(-r_3t) \quad (j = 1, 2, \text{ or } 3) \tag{7}$$

where the C's are determinable functions of the H's and the $-r$'s are roots of the determinantal equation

$$\begin{vmatrix} (r + k_{12} + k_{13} + k_{15}) & -k_{21} & -k_{31} \\ -k_{12} & (r + k_{21} + k_{23} + k_{25}) & -k_{32} \\ -k_{13} & -k_{23} & (r + k_{31} + k_{32} + k_{35}) \end{vmatrix} = 0$$

It has been shown (*10*) that $x_1(t)$ is of the form of Eq. (7). The experimental results that would be needed for the estimation of all the k's are neither available nor readily obtainable at this time. However, the derivatives of Eq. (6) can be approximated by the quotient of finite differences, $\Delta x_j/\Delta t$ ($j = 1, 2$ or 3), and one can then see from the data of Shemin and Rittenberg (*4*) that at least some of the interaction k's are of the same order of magnitude as the overall turnover k's (k_{j5}, or $1/T_j$).

Some of the interactions probably also involve one or more intermediates that can be transported by the blood. For example, this is likely to be the case in the carcass-internal organ protein interaction.

More detailed results can be obtained for the plasma proteins by using London's curves of N^{15}-tagged plasma (*5*), together with Dole's values for the com-

position of normal plasma (*11*). The isotopic excess in each plasma component was found to decline exponentially toward the average isotope concentration. In particular, the fractions of the plasma component isotopic excesses which interact daily are estimated at about 0.05, 0.5, 0.09, 0.06, and 0.09 for albumin, α-globulin, β-globulin, γ-globulin, and total plasma protein, respectively. Of the 0.09 value for total plasma protein only $1/T_1$, or about 0.036, could be attributed to plasma protein replacement from newly consumed protein.

On injecting dogs with tagged plasma protein, Yuile, Lamson, Miller, and Whipple obtained evidence of similar quick uptakes and subsequent interactions (*12*).

Also, the results estimated here for the various plasma components serve to illustrate the fact that all the other estimates are only averages of quantities having widely varying components.

Probably all the proteins that undergo the described interactions, as well as the relatively small amounts of nonprotein nitrogen, are the main constituents of the so-called nitrogen pool of the body.

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Manuscript received August 5, 1953.

Comments and Communications

The Phospho-Di-Anhydride Formula and Its Relation to the General Structure of the Nucleic Acids

SINCE the publication of the author's originally proposed phospho-tri-anhydride structure for the nucleic acids (*1*), Markham and Smith (*2*) have reported that both mono- and di-nucleotides possessing cyclic anhydride linkages and phospho-di-ester dinucleotides were products of the enzymatic degradation of yeast ribonucleic acid. Further, Cohn and Volkin (*3*) have succeeded in demonstrating the presence of 5-phospho-nucleotides in the digest of calf liver ribonucleic acid, which in itself is not incompatible with the phospho-tri-anhydride structure. More recently, Merrifield and Woolley (*4*) have presented conclusive evidence for the presence in yeast ribonucleic acid of "dinucleotides having two phospho-sugar ester bonds on one nucleoside and dinucleoside phosphates which have two nucleosides attached to one phosphate." These authors, aware only of the tri-anhydride structure, have concluded that their results ". . . do not seem to conform to the structure recently proposed by Ronwin." This conclusion is justified only when consideration is narrowed to the special case of the phospho-tri-anhydride structure (*1*); however, the results of these authors, together with those of the others mentioned above, are quite compatible with the amplified form of the P-O anhydride polymer core formula that is being presented here.

In addition, the author has recently become aware of the earlier synthesis of compounds possessing five oxygen atoms bound to a single phosphorus atom (*5*), which provides a precedent for the anhydride types that are proposed. The amplification is assisted diagrammatically by Fig. 1.

Section A is essentially one-half a unit cell for a phospho-di-anhydride structure for the nucleic acids along the same pattern as the previously proposed phospho-tri-anhydride structure (*1*). It is to be noted that all the nucleosides are depicted as engaged in diphospho-ester linkages that can give rise to such products as described by Merrifield and Woolley (*4*) and Markham and Smith (*2*). Each phosphorus atom carries one primary dissociation. Were the bond, P_2-O-P_3 of Sec. A, Fig. 1 hydrated, there would result two secondary dissociations in addition to the four primary dissociations already shown. This condition would split the P to O polymer core, but the chain would still be intact. Though the bases are pictured as parallel, but not coplanar to the sugar moieties (only the nuclei are shown), in accordance with Astbury's description (*6, 7*), they may be and probably are perpendicular to the D-ribose rings as recently reported for cytidine by Furberg (*8, 9*).

All the half-cells of any nucleic acid (either the ribo- or desoxyribo types) may possess the structure shown in Sec. A, Fig. 1 (the phospho-di-anhydride type) or they may all conform to the originally proposed phospho-tri-anhydride formula (Sec. B, Fig. 1) in which all the nucleosides are mono-esterified. As another condition, they may all assume the phospho-di-anhydride core wherein the nucleosides are bound as cyclic anhydrides (*2*) as depicted in Sec. C, Fig. 1. In this case, there is illustrated a situation in which three primary and one secondary dissociations are present for four P atoms. Another type of di-anhy-