phenylserine mixtures implies the entrance of both of the amino acids into the bacterial cells rather than the selective exclusion of phenylserine by tyrosine.

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Bony Mechanism of Automatic Flexion and Extension in the Pigeon's Wing

In 1875 Garrod (*1*) noted that Bergmann, and later Strauss-Durckheim, had earlier described a mechanism for automatic flexion and extension in birds' wings. Garrod also credited Alix for an observation of the same thing, reported in 1874 in Paris. Coues (*2*) further described the device. The function was suggested from gross examination of dissections, and no experimental evidence was offered. Since the date of these papers, no further research on this apparatus has been published.

Briefly, the theory of operation was that the radius and ulna move in a fashion similar to a pair of "drawing parallels." The proximal end of the radius butts against the humerus, while the distal end is in contact with the fused carpals (scapholunar), which in turn are in contact with the anterior (digit II side) surface of the carpometacarpal articulation (Fig. 1). Humeroulnar flexion forces the radius distally, and the radius forces the manus into a flexed position,

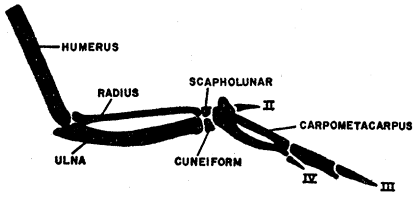

Fig. 1. Diagram of bones in pigeon's wing.

using the distal end of the ulna and the fused posterior carpal bones (cuneiform) as the fulcrum. Extension at the humeroulnar joint, through ligamentous attachments between the various bones, pulls the radius proximally and drags the manus into an extended position (with the same distal fulcrum as in flexion).

Experiments were devised to test these views of bone movement in the bird's wing. In two domestic pigeons, *Columba livia,* all flexors of the manus were cut; the birds were under general anesthesia, and it was a simple matter to cut all flexor tendons at the "wrist." After recovery from the operation, these birds flew in near-normal manner, but when they were perched the manus seemed to droop (extend) slightly. After 1 month, flight was so nearly normal that it was difficult to distinguish experimental birds from others in the room.

Cutting of the extensor tendons to the manus caused some further loss of delicate control, but the birds soon were able to fly. Eventually, these birds that had no muscular control of the hand flew easily, but they were always distinguishable in their awkwardness.

In two other pigeons, surgery was used to shorten the radius and prevent its simultaneous contact with the humerus and scapholunar. The left and right forewings were entered dorsally and a 5-millimeter section of the radius was sawed from the middle of the length of this bone. After insertion of a bone pin in the marrow cavity, the cut ends of the radius were pulled together with metal sutures. After recovery, the birds could fly, and wing-action was nearly the same as before the operation.

The flexor tendons for the hand were then cut. The birds could not fly, and the manus remained in an extended position at all times. Further surgery, to cut the extensor tendons, caused the hand to dangle, without visible control.

An attempt was subsequently made in each wing to remove the bone pin and insert a longer one to bring the radius back to its normal length. This was successful only on one side of one of the two birds; in the three other sites of bone shortening there had been too great a deposit of calcium on the outside of the bone to permit further surgery. The manus of the one wing in which the length of the radius had been restored was flexed and extended as the bird jumped from perch to perch.

At autopsy it was found that the radius had been shortened only 3 millimeters in one instance and 4 millimeters in the other two; normal length in the radii, as measured from x-ray photographs, was 48 millimeters.

It thus seems evident that flexion and extension of the manus can be accomplished without contraction of muscles extending to the manus. Flexion and extension at the humeroulnar joint, through action of the muscles of the upper arm, causes the same actions at the wrist.

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Liquid Scintillation Counting of C^{14}- and H^3-Labeled Amino Acids and Proteins

The method for tritiation of organic compounds described by Wilzbach (*1*) makes it possible to prepare with relative ease a wide variety of labeled compounds of high specific radioactivity. The applicability of the method in the labeling of intact protein molecules has now been demonstrated (*2*). Although the labeling procedure leads to degradation of a portion of each sample, a good yield of native protein of high specific radioactivity is obtained, as is evidenced by chromatographic homogeneity and retention of full enzymatic activity.

Effective use of these compounds in biological studies depends on the availability of a simple and sensitive system for radioassay. This is provided by the liquid scintillation counting technique. But, because the phosphors used in this system are nonpolar and are generally incorporated into an organic solvent (*3*), significant quantities of highly polar substances such as amino acids and proteins cannot be dissolved in the phosphor solution in unmodified form. A method for suspending such material in scintillating gels has been described for use in counting C^{14}-labeled compounds (*4*), but this is not applicable to the assay of tritium.

Passman *et al.* (*5*) have reported the use of a methanolic solution of the hydroxide form of a quaternary amine, Hyamine (*6*), to form a complex with $C^{14}O_2$ which is soluble in organic solvents, thus making it possible to dissolve up to 5 mmole of $C^{14}O_2$ in 35 ml of phosphor-containing toluene. As is described in the next paragraph, this same quaternary amine hydroxide (and several other quaternary amine hydroxides —for example, choline and tetraethyl ammonium hydroxide) can also complex amino acids and intact proteins. The phosphor dissolved in toluene can then be added to the methanolic solution of protein-amine complex, providing a clear

homogenous solution for assay in the scintillation counter.

The sample of protein or amino acid to be dissolved is weighed directly into the glass vial to be used for counting (7), and to it is added 1 ml of 1*M* Hyamine in the hydroxide form in methanol prepared according to Passman *et al.* (4). After the sample is completely dissolved, 10 ml of 600 mg percent diphenyloxazole (DPO) in toluene is added, and the contents of the vial are mixed.

Alanine, leucine, phenylalanine, tyrosine, and tryptophan are each soluble to a concentration of at least 20 mg/ml of Hyamine solution. Aspartic acid is somewhat less soluble than the afore-mentioned amino acids, and as little as 5 mg of arginine cannot be completely dissolved in 1 ml. It has been found that up to 10 mg of the following crystalline proteins dissolve readily at 37°C: insulin, ribonuclease, lysozyme, ovalbumin, and bovine serum albumin. Mixed tissue proteins [precipitated and washed with trichloroacetic acid (TCA), followed by ethanol-ether (1/1) and ether] dissolve with more difficulty, but 10 mg can be taken up in 1 ml of the amine solution by capping the vials and heating to 55° to 60°C for 1 to 2 hours.

After solution is complete and the vials have cooled to room temperature, 10 ml of DPO in toluene is added, as with the other samples. The vials are then cooled to the temperature of the counting chamber (−10°C). Samples of protein or amino acid containing salt (such as are obtained from column chromatography) can be taken up in the Hyamine solution satisfactorily, even though the salt does not dissolve.

With the exception of insulin, the afore-mentioned crystalline proteins do not cause quenching in amounts up to 10 mg per sample, and the counting efficiency with this method is approximately 5 percent. Insulin causes a decrease in the observed counting rate for tritium of from 14 to 57 percent as the amount of insulin is increased from 5 to 20 mg. Quenching is marked with the TCA-precipitated tissue proteins. Solutions of these denatured proteins frequently have a yellow-brown color. The intensity of the color varies with protein preparation, concentration of protein, and time of heating. The relationship between amount of protein and observed counts per minute is not linear for these preparations. As is shown in Table 1, the magnitude of this quenching effect can be determined by adding a standard amount of tritium-containing compound to all of the sample vials and recounting them. By means of the ratio of the increment in counting rate owing to the addition of standard for each vial to the counting rate for the standard alone, the counting rates of the unknown samples can be corrected for quenching. The corrected counting rates are linearly related to the amount of tritiated protein in the range from 2 to 10 mg. This method also corrects for differences in quenching that result from variable losses of methanol during heating and for variation in the counting vials. Because the intensity of color of the solution in the vials tends to increase on standing, it is important to make the quenching correction shortly after the sample is counted.

Table 1. Correction for quenching effect of trichloroacetic acid-precipitated protein.

Protein weight (mg)	Observed count/min minus background	Specific activity observed (count/min mg)	Increment in count/min owing to addition of standard*	Specific activity corrected for quenching (count/min mg)†
2	3933	1966	12081	2685
4	6658	1664	11260	2440
6	8295	1382	9578	2383
8	9523	1190	7613	2581
10	10685	1068	6722	2620

* Standard = 16,500 count/min of tritium-labeled Δ4-cholestenone.

† Observed count/min − background $\times \frac{16{,}500}{\text{increment in count/min owing to addition of standard}} \div$ sample weight

In the course of these studies, it has been noted that vials containing only methanol, Hyamine, toluene, and DPO give spuriously high counting rates (up to 500 count/min) when warm. As the temperature of the vial falls to that of the counting chamber, the counting rate returns to the normal background level (about 35 count/min). This phenomenon is observed only at the high voltages used for counting tritium. It does not occur when Hyamine is omitted from the solution—that is, with toluene and DPO alone—although it is evident when other quaternary amines are substituted for Hyamine. This effect of temperature is also observed when Hyamine is used with DPO in dioxane rather than in toluene solution.

A simple method for preparing samples of tritium or C^{14}-labeled amino acids and proteins for radioassay in the liquid scintillation counter has been described here. It will be evident that there are many possibilities for modifying the composition of the counting solution. For example, microgram quantities of ribonuclease dissolved in 0.5 ml of formamide can be counted satisfactorily after the addition of a solution of DPO in dioxane. However, the method that has been outlined would appear to be satisfactory for many purposes, and thus far none of the variations explored have been found to be of particular advantage. The ability to assay tritium directly in amino acids and proteins, coupled with the ease of labeling these compounds or their precursors with tritium by the Wilzbach technique (2) should provide a new tool for application to studies of protein synthesis and metabolism.

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Preparation of Tritiated Proteins by the Wilzbach Method

Wilzbach has recently described a simple procedure for introducing tritium into organic compounds (1). The compound to be labeled is exposed to 5 to 10 c of carrier-free tritium gas for several days at room temperature. Toluene, cholesterol, and digitoxin were labeled and found, on purification, to have specific radioactivities of 22.2, 64.3, and 90 mc/g, respectively. The labeling procedure caused some degradation, but this was considerably less extensive than that observed with the triton-recoil method (2). In view of the mild conditions used, it appeared possible that the method could be applied to the labeling of proteins. Preliminary results on the tritiation of lysozyme and ribonuclease by the Wilzbach method are described here.