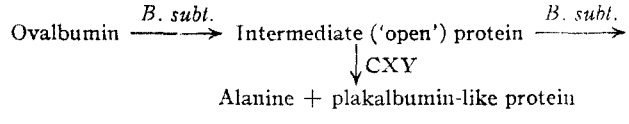
findings of the Carlsberg group.[13] The following scheme is proposed



Whether the initial reaction involves the opening of a peptidic bond or only represents a configurational alteration has not been determined.

Three of the CXY preparations used in these studies were found to be contaminated with bacteria (aerobic culture on yeast extract agar). The fourth preparation, however, showed no growth although it gave similar results with ovalbumin. Still the absence of viable bacteria does not prove the absence of enzyme.

Even though the *B. subtilis* enzyme so closely mimics the action of the contaminant, the possibility that a different proteolytic enzyme, possibly of pancreatic origin, is the real contaminant must be considered.[14] In any case the importance of recognizing such possible contaminants when using CXY in studies on proteins or peptides is clear. Very low levels of contamination may, as in this case, alter the observed reaction markedly.

A more detailed report will appear in *Comptes rendus du Laboratoire Carlsberg*.

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RECEIVED JULY 27, 1953

(13) K. Linderstrom-Lang, *Lane Medical Lectures*, **6**, 73 (1952).

(14) The possibility that CXY itself is able to "open" the ovalbumin molecule and that only this function is inhibited by DFP, while not ruled out, seems unlikely.

(15) Section on Cellular Physiology, National Heart Institute, Bethesda, Md. The author is deeply indebted to Professor K. Linderstrom-Lang and to Professor H. Neurath for their counsel.

THE SEPARATION OF THE FOUR PLATINUM GROUP METALS PALLADIUM, RHODIUM, IRIDIUM AND PLATINUM

Sir:

The separation of the four platinum group metals palladium, rhodium, iridium and platinum has been accomplished very simply at room temperature by the use of Dowex-50 cation exchange resin. Acting on some preliminary observations on rhodium by C. I. Browne (private communication) we have found that a mixture of these elements may be separated as follows: the solution is taken near dryness repeatedly with a mixture of nitric and perchloric acids until every trace of halide ion is removed and the ions are left in a small volume (0.2–0.5 ml.) of fuming perchloric acid. The solution is then diluted to a volume of approximately ten milliliters with distilled water and is run into the top of an ion-exchange column packed with Dowex-50 resin. Under these conditions platinum will pass through the column while the other three elements adhere. The column may be washed with distilled water to remove the last traces of platinum. If any halide ions remain in the solution, the platinum fraction will contain small amounts of the other three elements.

Palladium is then stripped from the column with dilute (0.05 to 0.5 *M*) hydrochloric acid. This process occurs quite readily.

Rhodium elutes gradually from the column with 2 *M* hydrochloric acid. This process is rather slow. Experiments are in progress to determine the effect of raising the temperature of the eluting solution to increase the rate of the reactions involved.

Iridium is removed with 4 to 6 *M* hydrochloric acid. This process, like the elution of rhodium, is rather slow.

Our experiments appear to show that sulfate ion prevents the adsorption of rhodium and iridium by the resin, presumably by forming neutral or anionic complexes with the metal cations.

This separation has been used in conjunction with other simple chemical steps for the radiochemical separation of pure rhodium in good yield from uranium fission products.

Further research will be done on this process.

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RECEIVED SEPTEMBER 3, 1953

PEROXIDE-INDUCED ADDITIONS OF METHYL FORMATE TO OLEFINS

Sir:

Preliminary studies with the peroxide-induced reaction of ethyl formate with ethylene gave complex products which contained both the ethyl esters of telomeric aliphatic acids, $H-(CH_2CH_2)_x-COOC_2H_5$, and formate esters of telomeric secondary alcohols, $H-COOCH(CH_3)-(CH_2CH_2)_x-H$. Methyl formate was successfully used in the work reported here to reduce to a minimum products of the latter type, and telomeric methyl esters, $H-(CH_2CH_2)_x-COOCH_3$, have been obtained.

A solution of *t*-butyl peroxide (8.8 g., 0.061 mole) in methyl formate (600 g., 10.0 moles) was held at 130° in a glass-lined, stainless steel autoclave under a pressure of ethylene (methyl formate vapor pressure plus ethylene, 340–440 p.s.i.) until its absorption ceased (20 hours). Distillation of the reaction mixture gave unreacted methyl formate and a higher boiling product (70–80 g.). Its further distillation through a small fractionating column packed with a wire spiral gave the following products: (1) a series of fractions containing *t*-butyl alcohol and methyl propionate (5.78 g., b.p. 72–84°, $n^{20}D$ 1.3761–1.3827, m.p. of *p*-toluidide 123°)[1,2]; (2) methyl valerate (6.91 g., b.p. 130°, $n^{20}D$ 1.3980, m.p. of *p*-toluidide 69–69.5°)[2]; (3) methyl enanthate (7.94 g., b.p. 113–116° at 100 mm., $n^{20}D$ 1.4165, m.p. of *p*-toluidide 79.5–80°, m.p. of mixture with an authentic sample gave no depression, enanthic acid made by carbonation of *n*-hexyl-magnesium bromide); (4) methyl pelargonate (6.24 g., b.p. 88–91° at 8 mm., $n^{20}D$ 1.4262, m.p. of *p*-toluidide 83.5°)[2]; (5) methyl hendecanoate (5.02 g.,

(1) All toluidides were prepared by the method of C. F. Koelsch and D. Tenenbaum, THIS JOURNAL, **55**, 3049 (1933).

(2) R. L. Shriner and R. C. Fuson, "Identification of Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., 1948, p. 222.

b.p. 70–72° at 1 mm., $n^{20}D$ 1.4349, m.p. of *p*-toluidide 79°)[2]; and (6) methyl tridecanoate (6.69 g., b.p. 90–95° at 1 mm., $n^{20}D$ 1.4405, m.p. of *p*-toluidide 87°, m.p. of mixture with authentic sample 87°).

Anal. Calcd. for $C_{20}H_{33}ON$: C, 79.15; H, 10.96; N, 4.62. Found: C, 78.85; H, 11.02; N, 4.73.

Tridecanoic acid for comparison was prepared by the reaction of carbon dioxide with *n*-dodecylmagnesium bromide. After the distillation of the above products, a residue (33.84 g., average mol. wt. 397, 12 ethylene : 1 methyl formate) remained.

A reaction mixture containing methyl formate (540 g., 9.0 moles), 1-hexene (25.2 g., 0.30 mole) and *t*-butyl peroxide (4.4 g., 0.031 mole) was held at 130–135° in the above apparatus for 24 hours. After the removal by distillation of unreacted methyl formate, 1-hexene and *t*-butyl alcohol, the following fractions were obtained: (1) methyl enanthate (4.71 g., b.p. 112–115° at 100 mm., $n^{22}D$ 1.4100, m.p. of *p*-toluidide 79.5–80°, m.p. of mixture with authentic sample 79.5–80°); (2) a mixture of 1:1 and 2:1 products (1.02 g., b.p. 120–130° at 20 mm., $n^{22}D$ 1.4303, average mol. wt. 188); (3) mixture as in 2 (1.23 g., b.p. 130–149° at 20 mm., $n^{22}D$ 1.4323); and (4) 2:1 product, presumably methyl β-*n*-butylpelargonate (2.34 g., b.p. 135–140° at 12 mm., $n^{22}D$ 1.4372, mol. wt. 236, calcd. mol. wt. 228). A residue (10.3 g., average mol. wt. 385) remained.

Further work to determine the scope of this reaction and other inducing agents for it is continuing.

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RECEIVED JULY 27, 1953

STUDIES ON PITUITARY ADRENOCORTICOTROPIN. VII. A C-TERMINAL SEQUENCE OF CORTICOTROPIN-A

Sir:

Corticotropin-A[1] has been treated with carboxypeptidase and the release of amino acids has been followed by paper chromatography. In a preliminary experiment, a sample of Corticotropin was incubated at 37° with 1% by weight of carboxypeptidase[2] for 24 hours in *p*H 7.5 ammonium acetate buffer. Acetic acid was added to stop the reaction and the buffer was removed by lyophilization. The residue was divided into two parts and chromatographed in the two solvent systems which we employ to resolve the principal amino acids.[3] Only phenylalanine, glutamic acid and leucine were detected. In order to determine the sequence of these amino acids, another sample of Corticotropin-A was incubated with carboxypeptidase under the same conditions and aliquots were removed at suitable intervals. These aliquots were chromatographed in one of the solvent systems (*s*-butyl alcohol/3% ammonia) in comparison with standard quantities of the three amino acids. By photometric measurement of the ninhydrin colors,[4] quantitative values were obtained. The results are shown in Fig. 1. It is apparent that the order of release is: phenylalanine, glutamic acid, leucine. Thus, in the nomenclature of Sanger,[5] the C-terminal sequence appears to be: . . Leu.Glu.Phe.

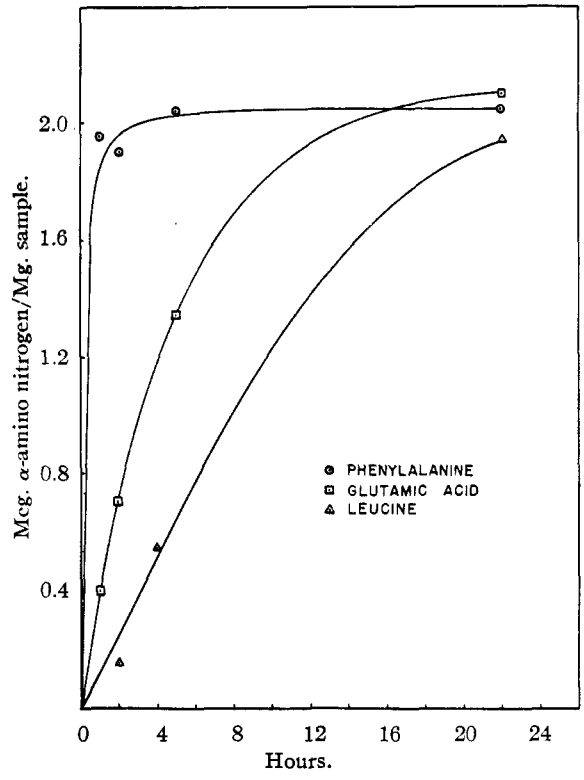

Fig. 1.—Rate of liberation of amino acids from corticotropin-A by treatment with carboxypeptidase.

Additional evidence for this sequence has been obtained from a study of three of the peptide fragments resulting from peptic[6] hydrolysis of Corticotropin-A. Table I shows the data which have been obtained on these fragments. In the first two columns are given the rates of movement in the two solvent systems used to separate the fragments.

TABLE I

THREE FRAGMENTS ISOLATED FROM PEPTIC DIGESTS OF CORTICOTROPIN-A

Rates of movement			
Partridge system[a]	*s*-Butyl alcohol ammonia	Isatin test	Amino acid composition (complete acid hydrolysis)
0.81	Tyr	+	Pro, Leu, Glu, Phe
.73	Thr	+	Pro, Leu, Glu
.68	Phe	−	Phe

[a] *n*-Butyl alcohol–acetic acid–water (80:20:100).

(1) W. F. White, THIS JOURNAL, **75**, 503 (1953).

(2) A six-times recrystallized material prepared by Miss E. Dickinson of The Armour Laboratories. Although free from chymotryptic contamination, the enzyme was incubated with di-isopropyl fluorophosphate before use.

(3) Phenol (72%)/water and *s*-butyl alcohol/3% ammonia, the latter used in an extended run with an absorbent pad at the bottom.

(4) Measurements were made with a photoelectric transmission densitometer (Densichron, W. M. Welch Mfg. Co., Chicago, Illinois). Amino acid standards were run at levels of 0.1, 0.25 and 0.4 μg. α-amino nitrogen.

(5) "Advances in Protein Chemistry," Vol. VII, Academic Press, Inc., New York, N. Y., p. 5.

(6) The pepsin treatment was done at 37° with 1% enzyme (pepsin crystallized Armour) in 0.1 *N* formic acid (*p*H 2.2–2.3). The fragments described appear in both 6-hour and 24-hour digests in addition to other fragments not mentioned in this paper.