to 10^{-2} molar). It is proposed here that Tl^+ increases the rate of Ce^{++++} reduction through reaction with OH radical to form Tl^{++} which reduces Ce^{++++} according to the following sequence of reactions.

$$Tl^+ + OH \longrightarrow Tl^{++} + OH^- \quad (1)$$

$$Tl^{++} + Ce^{++++} \longrightarrow Tl^{+++} + Ce^{+++} \quad (2)$$

According to this mechanism, the 100 ev. yield of Ce^{+++} increased by Tl^+ should be equal to $2G_{H_2O_2} + G_H + G_{OH}$. The previously reported[3] values for $G_{H_2O_2}$, G_H and G_{OH} of 0.78, 3.70 and 2.92, respectively, would predict a value for $2G_{H_2O_2} + G_H + G_{OH}$ of 8.18. The difference between 8.18 and 7.85 is real and may indicate the importance of reaction 3

$$OH + H_2O_2 \longrightarrow H_2O + HO_2 \quad (3)$$

in regions of high ionization density as reported by Hart.[4]

NO_3^- also increases the rate of Ce^{++++} reduction in 0.8*N* sulfuric acid. It has been proposed[5] that in nitric acid solutions the reduction of Ce^{++++} is increased through reaction of OH radical with NO_3^-. Figure 1 shows that, in 0.8*N* sulfuric acid, NO_3^- increases the 100 ev. yield of Ce^{+++} when Tl^+ is present by the same amount as when Tl^+ is absent. This is interpreted as conclusive experimental evidence that the increase in 100 ev. yield of Ce^{+++} by NO_3^- is not attributable to OH radical reactions since the OH radicals are being efficiently utilized by Tl^+. If NO_3^- reacts with the H and OH radicals in a manner similar to that reported[6] for NO_2^-, the products must react with Ce^{++++} and Tl^+ in a manner equivalent to H and OH.

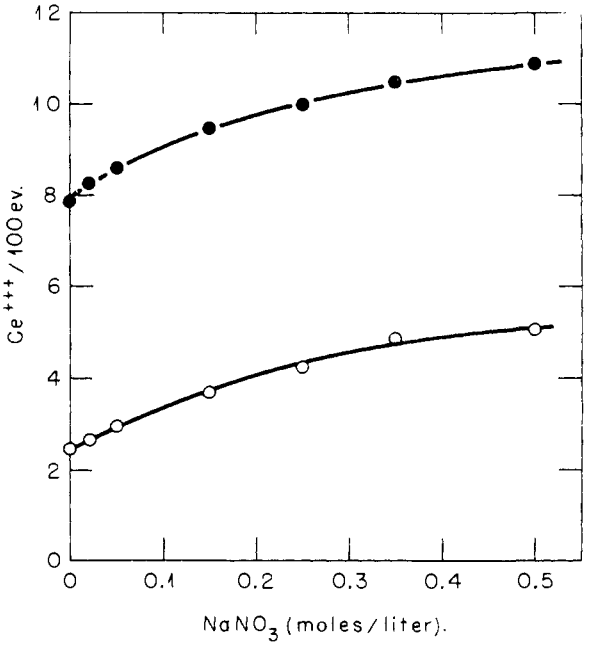

Fig. 1.—Effect of $NaNO_3$ on the reduction of ceric ion in 0.8 *N* sulfuric acid solutions; Ce^{++++} concentration in all solutions was initially 4×10^{-4} molar: ○, Tl^+ absent; ●, 2×10^{-3} molar Tl^+.

(3) T. J. Sworski, THIS JOURNAL, **76**, 4687 (1954).
(4) E. J. Hart, *Radiation Research*, **2**, 33 (1955).
(5) G. E. Challenger and B. J. Masters, THIS JOURNAL, **77**, 1063 (1955).
(6) H. A. Schwarz and A. O. Allen, *ibid.*, **77**, 1324 (1955).

An alternative mechanism for the effect of NO_3^- is the direct action of gamma radiation on NO_3^-. This direct action is considered as resulting from excitation of the NO_3^- with resultant decomposition to NO_2^- as in the photochemical reduction[7] of NO_3^-. Ce^{++++} is then reduced by NO_2^-. NO_3^- excitation may result from the action of subexcitation electrons[8,9] or from the direct transfer of excitation energy from the excited water molecules (before collisional deactivation) to NO_3^-.

(7) E. Warburg, *Sitzb. Preuss. Akad., Math.-Phys. Kl.*, 1228 (1918); *Chem. Abs.*, **14**, 1930 (1920).
(8) J. Weiss, *Nature*, **174**, 78 (1954).
(9) R. L. Platzman, *Radiation Research*, **1**, 558 (1954); **2**, 1 (1955).

CHEMISTRY DIVISION
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OAK RIDGE, TENNESSEE

RECEIVED AUGUST 8, 1955

THE USE OF PERFLUOROCARBOXYLIC ACIDS AS SEPARATING AGENTS[1]

Sir:

We have found that the perfluorocarboxylic acids[2] are useful extracting and separating agents for a wide variety of cations in aqueous solution. Using ethyl ether as the second solvent the extraction is found to depend on (a) the *p*H of the aqueous layer; (b) the ionic charge on the cation; (c) the nature of the anions present. Using perfluorobutyric acid as the extracting agent, trivalent cations, *e.g.*, Fe(III), Al(III), Cr(III), and a few divalent cations, UO_2(II), Be(II) can be effectively separated from monovalent and other divalent cations. With perfluoroöctanoic acid as the extracting agent, divalent cations, *e.g.*, Ca(II), Mg(II), Fe(II), Pb(II), Zn(II), etc., can be separated from monovalent cations.

It is generally found that the extraction proceeds best at a *p*H just less than that at which the cation would form an insoluble hydroxide. In line with this observation it is found that the extracted species is a basic salt.[3] Analyses of crystalline products obtained on evaporating the ether extracts indicate formulas such as $Fe(OH)(C_3F_7COO)_2 \cdot 2H_2O$; $Al(OH)(C_3F_7COO)_2 \cdot 2H_2O$; $Be(OH)(C_3F_7COO) \cdot 5H_2O$.

The separation factors for individual extractable species vary from 4–400 when the metal is dissolved in aqueous solution as the nitrate or perchlorate. Other anions will interfere with the extraction in some degree; fluoride and sulfate in particular are found to prevent extraction under conditions employed so far in this work. In the absence of interfering anions no salting agent is necessary although the separation factor obtained is, of course, influenced by the relative proportion of the perfluoroacid to the metallic ion.

While monovalent cations are not extracted,

(1) This work was done at the Oak Ridge Gaseous Diffusion Plant operated for the Government by Union Carbide and Carbon Corporation.
(2) These acids may be obtained from the Minnesota Mining and Manufacturing Company, Minneapolis, Minnesota. We wish to thank them for samples supplied us in the early stages of this work.
(3) The calcium (II) ion appears to extract as a normal salt and is an exception to this generalization.

certain of them, *e.g.*, bismuthyl ion, can be precipitated quantitatively by adding perfluoroacetic acid (at *p*H 0.5). In general, cations forming insoluble hydroxides can be precipitated in abnormal *p*H intervals by carrying out the precipitation in the presence of appropriate perfluoroacids. It is not yet known whether the perfluoroacid anion is combined in stoichiometric proportions in these precipitates.

K-25 TECHNICAL DIVISION
CARBIDE AND CARBON CHEMICALS COMPANY G. F. MILLS
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RECEIVED JULY 29, 1955

STUDIES ON ADRENOCORTICOTROPIN. XII. ACTION OF AMINOPEPTIDASE ON CORTICOTROPIN-A; EFFECT ON BIOLOGICAL ACTIVITY

Sir:

In previous communications from this laboratory, it has been shown that corticotropin-A is a straight chain polypeptide containing thirty-nine amino acid residues[1] with the N-terminal sequence: Ser.Tyr.Ser.Met.Glu.His.Phe.Arg.Try.... Our evidence for this sequence was based partly on chemical reactions on the intact molecule[2] and partly on a study of overlapping fragments produced by the action of endopeptidases on corticotropin-A.[3] In our hands, both of the chemical reagents used (dinitrofluorobenzene and phenyl isothiocyanate) and two of the endopeptidases (chymotrypsin and trypsin) attacked the molecule at several points simultaneously and therefore did not provide specific knowledge of the relationship between structure and physiological activity.[4] Pepsin, however, rapidly split three bonds near the C-terminus of corticotropin-A with no effect on activity and then slowly split the bond between glutamic acid in position 5 and histidine in position 6.[5] Serial application of the Sayers test indicated that the rupture of this bond was paralleled by a loss in physiological activity.

With the availability of highly purified aminopeptidase,[6] an opportunity was afforded of studying the effect of the serial removal of amino acids at the N-terminus of corticotropin-A. Five milligrams (approximately 1 micromole) of the purified hormone was incubated[7] with 2 units of a highly purified aminopeptidase preparation at 37° and aliquots were withdrawn at intervals over a 24-hour period. Quantitative amino acid determinations were made on portions of each aliquot by means of paper chromatography in the 2-butanol:ammonia system.[8] Figure 1 shows a plot of the amount of each amino acid released against the time of incubation. The situation is complicated somewhat by the presence of two serine residues in the sequence, but the results are in general agreement with the rates of hydrolysis for the individual amino acids as established by the University of Utah group with the corresponding amides.[9] The terminal amino acid, serine, is split very slowly (C_0 = 106) and therefore limits the rate of the more easily removed tyrosine (C_0 = 2,200). The second serine residue in position 3 again limits the more rapidly split methionine. Glutamic acid (position 5) apparently is also very slow and limits the next four amino acids, all of which have high rates. Glycine (position 10) is again very slow (C_0 = 18) and, due to the limited opportunity for reaction at this point, was not detected on our chromatograms.

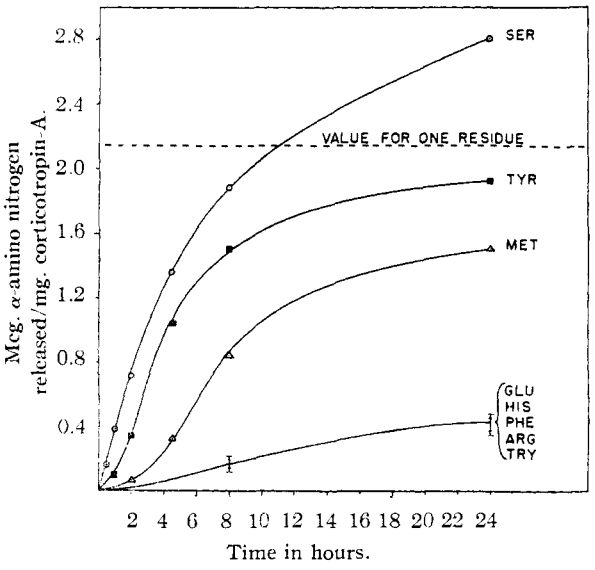

Fig. 1.—Release of amino acids from corticotropin-A by action of aminopeptidase. Since the corticotropin-A preparation contained salt, the value for one residue was an average value calculated from the amino acid composition after complete acid hydrolysis and taking into account the known number of residues of each amino acid.

In attempting to relate the release of amino acids to physiological activity, the 4.5-hour fraction was selected for assay. At this point a total of approximately two-thirds of a residue of serine had been released. Since the rate for tyrosine is about twenty times that for serine, the value for tyrosine can be taken as a measure of the amount of the first serine unit released, and the difference between the serine and the tyrosine values as the amount of the second serine unit released. Thus, at 4.5 hours more than one-half of both the first serine and the tyrosine had been removed. Assay of the 4.5

(1) W. F. White and W. A. Landmann, THIS JOURNAL, **77**, 1711 (1955).

(2) W. A. Landmann, M. P. Drake and W. F. White, *ibid.*, **75**, 4370 (1953).

(3) W. F. White and W. A. Landmann, *ibid.*, **77**, 771 (1955).

(4) Throughout this paper the terms "physiological activity" or simply "activity" refer to depletion of adrenal ascorbic acid, as measured by the subcutaneous method, U. S. Pharmacopeia, Vol. XV, p. 176.

(5) W. F. White, THIS JOURNAL, **74**, 4194 (1954).

(6) The preparation used in this work was kindly furnished by Dr. Emil L. Smith of The University of Utah College of Medicine. The method of preparation is described in *J. Biol. Chem.*, **212**, 255 (1955).

(7) The reaction was carried out in 0.005 *M* ammonium veronal at *p*H 8.5 and containing 0.0025 *M* magnesium chloride. The aminopeptidase preparation had been treated with diisopropylfluorophosphate at the time of preparation. A small amount of toluene was added to the mixture before incubation.

(8) For details of the quantitative chromatographic estimation of amino acids, *cf.* J. F. Roland, Jr., and A. M. Gross, *Anal. Chem.*, **26**, 502 (1954).

(9) E. L. Smith and D. H. Spackman, *J. Biol. Chem.*, **212**, 271 (1955).