TABLE I

ALKYL ESTERS OF ISODEHYDROACETIC ACID

Alkyl	Yield, %	B.p., °C.	Mm.	$n^{t}D$	t, °C.	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
Methyl	99	67[a]	....	....	....	...	...	...	...
n-Propyl	79	116	8	1.5093	23.5	62.85	62.64	6.67	6.80
i-Propyl	85	158	8	1.5050	24	62.85	62.74	6.67	6.80
n-Butyl	89	167	7	1.5063	23.5	64.22	64.27	7.15	7.37
i-Amyl	68	177	8	1.5032	23.8	65.47	65.20	7.56	7.62
Cetyl	71.5	55[a]	....	....	....	73.42	73.33	10.41	10.50

[a] Melting point.

Anal. Calcd. for $C_{11}H_{14}O_4$: C, 62.85; H, 6.67. Found: C, 62.64; H, 6.80.

Methyl isodehydroacetate was prepared from 1.0 g. (0.054 mole) of the acid chloride and 5 ml. of absolute methanol. Evaporation gave *ca.* 1.0 g. of the ester, m.p. 67°; reported m.p. 67–67.5°.[4] The product was converted to the 3-bromo derivative by reaction with an equivalent amount of bromine in carbon tetrachloride; m.p. 133–134°; reported m.p. 135°.[5]

(4) R. Anschutz, P. Bendix and W. Kerp, *Ann.*, **259**, 156 (1890).
(5) E. Buchner and H. Schröder, *Ber.*, **35**, 790 (1908).

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Studies on Pituitary Adrenocorticotropin. III. Differentiation of Three Active Types on XE-97 Resin

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By the use of the cation exchange resin Amberlite XE-97,[1] three active ACTH types have been differentiated in hog pituitary extracts. Two active types have been separated from the highly purified fractions of acid- and pepsin-treated extracts described in a previous publication.[2] A third active type has been found in highly potent oxycellulose eluates made from hog pituitaries[3] and in other non-hydrolyzed preparations.

Figure 1 shows a typical fractionation of an acid- and pepsin-hydrolyzed sample which had previously been purified by means of a chromatopile. The XE-97 bed[4] was equilibrated with 0.1 *M* sodium carbonate–bicarbonate buffer at *p*H 8.5 before application of the sample as a solution in the same buffer. The portion passing directly through the column at *p*H 8.5 (fraction I) was physiologically inert. However, successive substitution of *p*H 9.25 and 11.25 buffers eluted two additional fractions (II and III, Fig. 1) which showed activities in the range of 100 to 150 u./mg. of peptide.[5]

(1) Rohm & Haas Co., Washington Square, Philadelphia 5, Pa.
(2) W. F. White, W. L. Fierce and J. B. Lesh, *Proc. Soc. Exptl. Biol. Med.*, **78**, 616 (1951).
(3) E. B. Astwood, M. S. Raben, R. W. Payne and A. B. Grady, THIS JOURNAL, **73**, 2969 (1951).
(4) The resin was prepared as follows: The material as supplied by the manufacturer was stirred three or four times with water, each time allowing the suspension to settle four or five minutes and pouring off the fines. The washed resin was then cycled three times batchwise using *N* sodium hydroxide and *N* hydrochloric acid. After thorough washing with water, the resin was stored in the acid form either wet or dry until use.
(5) Fractions were assayed by the Munson modification of the adrenal ascorbic depletion method of M. A. Sayers, G. Sayers and L. A. Woodbury, *Endocrinol.*, **42**, 379 (1948). This technique has provisional USP approval. The samples were administered intravenously. All activities are expressed as USP units.

In other runs the range between *p*H 8.5 and 11.25 has been investigated in small increments without revealing additional components. The non-identity of fractions II and III has been proved by rerunning them individually through the same type of column, under which condition the integrities of the fractions were maintained.

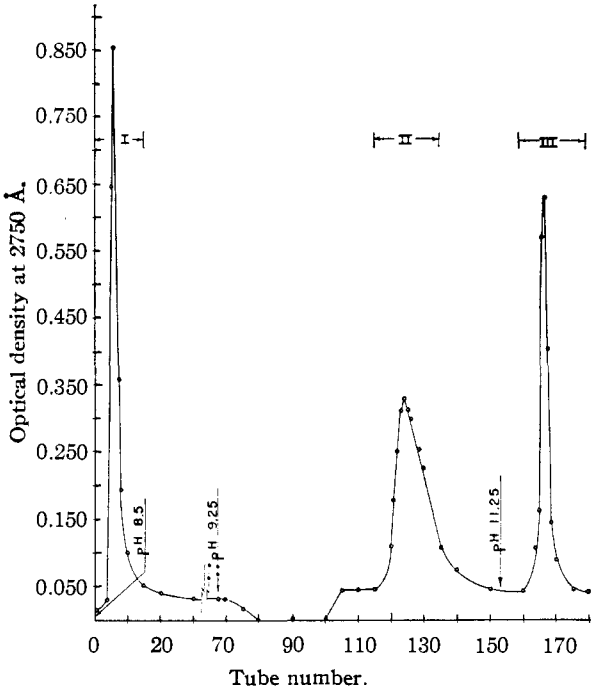

Fig. 1.—Chromatography of a highly purified acid- and pepsin-treated ACTH preparation on Amberlite XE-97 resin. The sample (10.5 mg. at 65 u./mg.) in 1 ml. *p*H 8.5 buffer was applied to a column 15 cm. high in a tube 0.9 cm. in diameter. Rate of flow was 0.5 ml./min. Volume collected per tube was 1 ml. The distribution of ultraviolet absorption in the fractions indicated was: I, 25%; II, 28%; III, 24%. The distribution of activity was: I, < 2%; II, 64%; III, 19%.

A much different result was obtained when highly active unhydrolyzed ACTH preparations were subjected to the XE-97 procedure. Here almost all of the ultraviolet absorbing material was eluted at *p*H 8.5 and the activity appeared to be associated with a minor component poorly separated from the major peak.[6] Very little ultraviolet absorbing material and no activity appeared either at *p*H 9.25 or *p*H 11.25. Increasing the height of the

(6) Dixon, *et al.*, *Nature*, **168**, 1044 (1951), noted a similar behavior with a crude unhydrolyzed preparation. However, their conditions of *p*H and buffer composition were different.

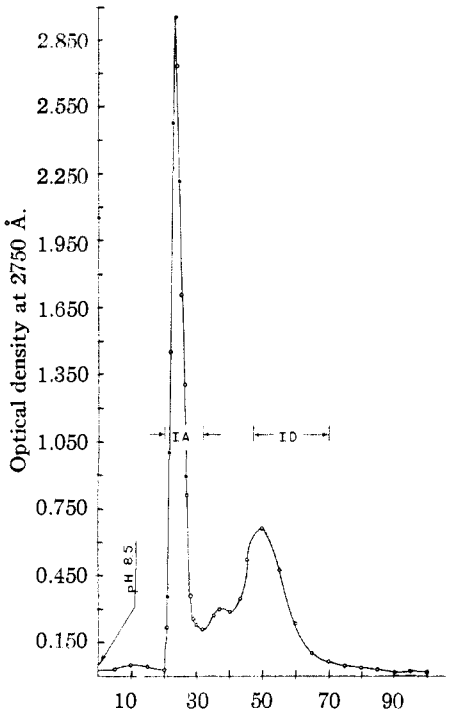

Fig. 2.—Chromatography of an oxycellulose eluate ACTH preparation on Amberlite XE-97 resin. The sample (430 mg. at 40 u./mg.) in 100 ml., *p*H 8.5 buffer was applied to a column 70 cm. high in a tube 5.4 cm. in diameter. Rate of flow was 3.5 ml./min. Volume collected per tube was 17 ml. The distribution of ultraviolet absorption was: IA, 54%; ID, 20%. The distribution of activity was: IA, < 2%; ID, 70%.

XE-97 column improved the resolution at *p*H 8.5. Figure 2 shows a run using a 70-cm. bed.

TABLE I

EFFECT OF VARIOUS HYDROLYTIC TREATMENTS ON THE PERFORMANCE OF AN OXYCELLULOSE ELUATE[a] ON XE-97 RESIN

Type of hydrolytic treatment	*p*H 8.5 (Type ID) O.D.[b]	Activity[c]	*p*H 9.25 (Type II) O.D.	Activity	*p*H 11.25 (Type III) O.D.	Activity
None	31	70	5	<5	17	<5
Pepsin[d]: 2 hours	None	<5	21	100	17	<5
Pepsin: 24 hours	None	<5	44	80	12	<5
Pepsin: 4 hours followed by acid (1 hour at 100° in 0.01 *N* HCl)	None	<5	27	75	21	25

[a] The starting material in the experiments summarized in this table was a typical oxycellulose eluate made by the Astwood process and having a potency of about 25 μ/mg. [b] Optical densities are given as percentages of the total optical density recovered in all fractions. The difference between the totals given in the three fractions listed and 100 is the amount appearing before fraction ID. [c] Given as percentages of the amount of activity put on the column. Where the totals do not add up to 100, it is assumed that the remainder was destroyed in handling. [d] In these experiments, the amount of pepsin was 1% of the ACTH fraction and the digestion was done at 37° in 0.01 *N* HCl (*p*H 2.1–2.3). In other experiments, a considerable variation (0.6–4%) in the percentage of pepsin was without appreciable effect on the results.

In studying the relationships between the three types of ACTH, an oxycellulose eluate has been treated with pepsin and with pepsin and acid. Table I shows the fractionation of these materials on XE-97 resin. The percentages of ultraviolet absorption and of activity going to the various positions are shown in each case. As seen in the table, treatment with pepsin for as little as two hours converts all the type ID activity to type II, while even 24 hours does not produce an appreciable amount of type III. However, subsequent treatment with acid converts at least part of type II into type III.

In view of the fact that the variations in the conditions of pepsin treatment in the experiments of Table I and of other experiments (*cf.* footnote *d*, Table I) include those used by Brink, *et al.*,[7] it would appear likely that Type II activity predominated in the concentrate from which Corticotropin-B was isolated. Our experiments, using XE-97 resin on material processed by successive pepsin and acid treatment, appears to be the first in which two hydrolyzed types of ACTH are clearly differentiated. Further work directed toward the isolation in pure form of the three active types is under way.

Acknowledgment.—The authors wish to acknowledge the technical assistance of Mr. R. L. Peters.

(7) N. G. Brink, F. A. Kuehl, Jr., J. W. Richter, A. W. Bazemore, M. A. P. Meisinger, D. E. Ayer and K. Folkers, THIS JOURNAL, **74**, 2120 (1952).

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Rates of Solvolysis of Some Alkyl Fluorides and Chlorides[1]

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Table I shows that the RCl/RF rate ratio for hydrolysis in neutral or slightly acidic solutions varies from 10^6 for triphenylmethyl (trityl) halides to less than 10^2 for benzoyl halides. This reflects the tendency of C–X rupture to be more complete than O–C formation at the transition state of trityl halide hydrolysis, and the opposite tendency with benzoyl halides.[2] The change from Cl to F hinders the C–X break, but facilitates O–C formation by making the carbon more electron-deficient and positive.

The ratio is further reduced in basic solution (*cf.* Table II). Toward hydroxide ion, benzoyl fluoride actually reacts faster (by 40%) than benzoyl chloride.

Experimental

Reagents.—Benzoyl fluoride was prepared from 140 g. (1 mole) of benzoyl chloride in a polyethylene bottle, fitted with copper entrance and exit tubes in a 2-hole rubber stopper, by passing in anhydrous hydrogen fluoride until the exit gas gave no precipitate with silver nitrate solution. Best results were obtained when the polyethylene bottle rested in an ice-bath and the hydrogen fluoride was con-

(1) This work was supported by the Office of Naval Research.

(2) The same factor is responsible for the negative ρ-values[3] and small s-values[4] generally observed with trityl halides in contrast to the positive ρ-values and large s-values with benzoyl halides.

(3) C. G. Swain and W. P. Langsdorf, Jr., THIS JOURNAL, **73**, 2813 (1951).

(4) C. G. Swain and C. B. Scott, *ibid.*, **75**, 141 (1953).