mutants of *S. typhimurium*[6] blocked before IGP are either unable to form compound III (strain hi-F-41) or are unable to convert it to IGP (strain hi-A-5).

According to these results purine nucleotides play a catalytic role in the biosynthesis of histidine: AMP donates an N-C fragment to RP for the formation of the imidazole ring of histidine and is concomitantly converted to AICAR; this compound in turn reacts with a single carbon unit to regenerate the purine ring.

(6) Kindly supplied by Dr. M. Demerec.

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A CADMIUM PROTEIN FROM EQUINE KIDNEY CORTEX

Sir:

Cadmium never has been demonstrated to be an integral part of a natural product although present in various species.[1,2,3] Physiological function has not been shown.

Colorimetric analyses[4] of human, horse, cow, hog, and sheep kidney for cadmium led to the choice of horse kidney cortex for fractionation.

Fractionation of horse kidney cortex with ethanol and ammonium sulfate gave a product containing 20 to 25 *milligrams* of cadmium per gram dry weight of trichloroacetic acid precipitable material in successive fractionations. Ultracentrifugation[5] in a synthetic boundary cell showed the final products of four successive fractionations to be monodisperse with a sedimentation constant (uncorrected for viscosity and diffusion) varying from 0.94 to 1.22×10^{-13}. Paper electrophoresis at *p*H 8.5 of the product of one fractionation showed three components moving toward the cathode, the slowest comprising about 70% of the total material.

The fractions were analyzed colorimetrically[4] and by emission spectrography[6] (Table I). The cadmium content rises throughout the fractionation, a 30-fold increase from the first extract to the product. Cadmium is not removed by dialysis at *p*H 7, but is by treatment with hot trichloroacetic acid. With the exception of zinc, the other metals present initially and introduced during fractionation are removed and are low in concentration in the final material. Isomorphism cannot be excluded as an explanation of the substantial, although lesser, increase of zinc content as fractionation proceeds, nor can it be ruled out that cadmium is associated with one and zinc with another of the three electrophoretic fractions.

The product contains 14% nitrogen, measured on the material precipitated by trichloroacetic acid. It reacts positively to the biuret and ninhydrin tests. Hydrolysis and paper chromatography showed serine, glycine, aspartic, and glutamic acids, among other amino acids not identified. The last fraction (Table I, Fraction VII) contains about 1% of hexoseamine.[7] A carbazole test for uronides[8] was negative.

There is no ultraviolet absorption maximum near 280 mμ at *p*H 7 or *p*H 12, indicating a low content of aromatic groups. Absorption bands have not been found in the visible region. The infrared spectrum of a potassium bromide pellet of the lyophilized product closely resembles those obtained for several proteins.[9]

The low sedimentation constant and high metal content of this material are indicative of a low molecular weight protein, probably containing a small number of cadmium atoms per molecule. Characterization of this unusual natural product is in progress.

TABLE I

EMISSION SPECTROGRAPHIC AND COLORIMETRIC ANALYSES OF HORSE KIDNEY CORTEX FRACTIONS

Cadmium determined by spectrography[5] and by colorimetry[4]; all other metals determined spectrographically. Protein measured by dry weight of material precipitated by trichloroacetic acid. Data expressed as μg./g. wet weight of cortex, and as μg./g. protein for the fractions.

	Cortex	Fraction I	Fraction II	Fraction III	Fraction IV	Fraction V	Fraction VI	Fraction VII
Preparation E: colorimetric cadmium	82.7	1130	3050	17,100	14,300	*a*	*b*	24,500
Preparation C: colorimetric cadmium	83.7	754	3440	8,900	9,990	17,100	21,600	22,400
Spectrographic cadmium	137	687	2630	9,730	*c*	*c*	20,700	24,200
Spectrographic zinc	91	340	1440	3,600	3,330	*c*	4,910	5,880
Other metals (spectrographic)[e]	829	2410	2200	4,910	3,190	45,200[d]	15,400[d]	2,470

[a] Protein concentration too small to measure by trichloroacetic acid precipitation. [b] Sample lost. [c] Sample size inadequate. [d] Contamination with Mg and Ca introduced with ammonium sulfate. [e] Mg, Ca, Ba, Sr, Al, Fe, Mn, Cr, Pb; Na, K, Cu not determined.

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(2) A. K. Klein and H. J. Wichmann, *J. Assoc. Off. Agric. Chem.*, **28** 257 (1945).

(3) A. O. Voinar, *Trudy Konf. Mikroelement* 1950, 580 (1952); *Akad. Nauk U.S.S.R.*, Translation R-J-296, of Associated Technical Services, East Orange, New Jersey.

(4) B. E. Saltzman, *Anal. Chem.*, **25**, 493 (1953).

(5) The ultracentrifugations were done by Mr. Paul M. Reilly of the Biophysics Research Laboratory.

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(10) Fellow of the Kettering Foundation, Dayton, Ohio.

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RECEIVED AUGUST 1, 1957

THE EFFECT OF SODIUM CHLORIDE ON THE *alpha*-CHYMOTRYPSIN CATALYZED HYDROLYSIS OF METHYL HIPPURATE

Sir:

The development of the *p*H-Stat[1,2] has made it possible to observe certain enzyme-catalyzed reactions in the absence of conventional buffers and to thereby determine the effect of added salts upon the rates of such reactions at a constant *p*H and at relatively low ionic strengths. In the course of such studies, it has been observed that the initial rates of the α-chymotrypsin catalyzed hydrolysis of methyl hippurate in aqueous solutions at 25.0° and *p*H 7.90 ± 0.01 and at various initial specific substrate concentrations are markedly dependent upon the presence of added sodium chloride. The nature of this dependency is most readily illustrated by a consideration of the separate dependencies of the constants K_S' and k_3' for the above reaction system upon the concentration of added sodium chloride. It will be seen from Fig. 1 that

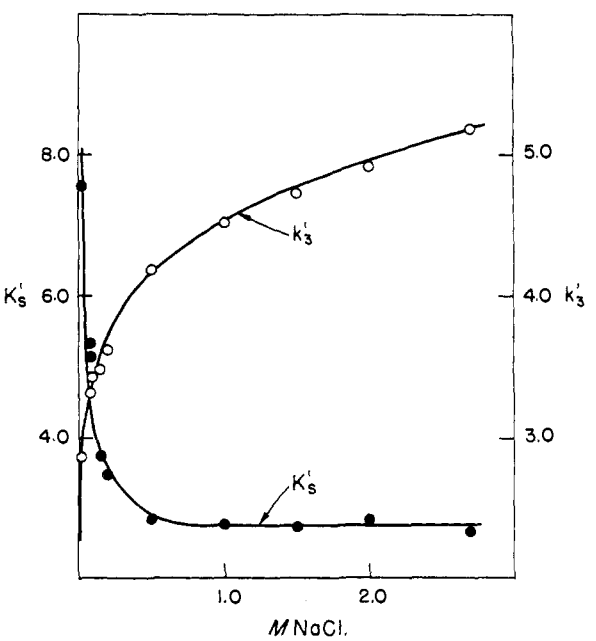

Fig. 1.—Dependence of K's and k'_3 upon concentration of sodium chloride; K's in units of 10^{-3} *M*, k'_3 in units of 10^{-3} *M*/min./mg. protein-nitrogen per ml.

at concentrations of sodium chloride greater than 1 *M* the value of K_S' is essentially constant but as the concentration of sodium chloride is decreased below 1 *M* the value of K_S' begins to increase, slowly and then rapidly, and as the system approaches zero ionic strength the value of K_S' tends to become very large. While the value of k_3' generally decreases with decreasing concentration of sodium chloride, at concentrations below 1 *M* the value of k_3' decreases more rapidly than at concentrations above 1 *M* and as the system approaches zero ionic strength the value of k_3 appears to become very small. Although it is not possible to conduct an experiment in a system of zero ionic strength, it may be inferred from Fig. 1 that in such a system the value of K_S may approach infinity and the value of k_3 may approach zero with the result that no reaction may be observed. The implied inertness of α-chymotrypsin in reaction systems containing no added sodium chloride may be a property of the protein molecule *per se*, or may be due to a transformation of the active enzyme to species that are incapable of combining with the specific substrate. In either case, the addition of sodium chloride leads to the formation of a more active enzyme.

Results similar to but not identical with those summarized in Fig. 1 have been obtained with methyl hippurate and other salts, *e.g.*, lithium chloride, potassium chloride, sodium bromide and magnesium chloride, and with sodium chloride and another ester type of specific substrate, *i.e.*, acetyl-L-valine methyl ester. Therefore, it should be realized that the case involving α-chymotrypsin, methyl hippurate and sodium chloride is not unique but is representative of the general behavior of a number of similar systems.

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THE CONVERSION OF RUSCOGENIN TO 1ξ-HYDROXYPROGESTERONE

Sir:

The isolation of ruscogenin,[1] a steroidal sapogenin from *Ruscus aculeatus L.*, and the recognition that this substance possesses the structure of diosgenin with an additional hydroxyl group,[2] furnished an interesting potential starting material for novel compounds related to physiologically active substances. We wish to report here the synthesis of 1ξ-hydroxyprogesterone.

While it was believed at first that ruscogenin has its second hydroxyl group at C-19,[2] the work of Burn, Ellis and Petrov,[3] as well as subsequent work by Lapin,[4] indicates the hydroxyl to be at C-1. In agreement with these authors, we believe the latter to be the case and wish to offer additional evidence. Authentic (25D)[5] ruscogenin diacetate,

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(1) C. Sannié, H. Lapin, F. Eloy and L. Cogolludo Sanchez, *Bull. soc. chim. Biol.*, **39**, 301 (1957), and references listed therein.

(2) C. Sannié and H. Lapin, *Bull. soc. chim. France*, 1552, 1556 (1955).

(3) D. Burn, B. Ellis and V. Petrov, *Proc. Chem. Soc.*, 119 (1957).

(4) H. L. Lapin, *Compt. rend.*, **244**, 3065 (1957).

(5) It is possible to isolate not only the 25D isomer, ruscogenin, but also a 25L compound, neoruscogenin, m.p. of the diacetate 139–141°, from *Ruscus* sapogenins. The two isomers are difficult to obtain completely free from one another. We are indebted to Dr. Lapin for this information.