and varying amounts of minor components. In the system studied the partition ratios of the major components of beef, pork and sheep insulins appeared to be identical within the experimental error, but this by no means proves that these components are identical, particularly with a solute of the size and complexity of insulin. It indicates only that their physical properties are remarkably similar. From the standpoint of a critical evaluation of the experimental methods available for separating intact proteins and in order to find out whether or not the purity of a given preparation can be established with any degree of reliability by countercurrent distribution, it is important to compare these purified A components from the different species in the most searching manner. We have therefore compared their quantitative amino acid compositions by ion exchange chromatography.[2] Striking differences have been found.

Analyses of products from hydrolyses carried out for 24 hours have shown that there are six amino acids which are present in different amounts in insulins prepared from the three species mentioned. These are given in Table I. Otherwise the amino acid compositions of the three preparations were identical. Minimum molecular weights of 5734, 5778 and 5704 were calculated for beef, pork and sheep insulins, respectively, from the amino acid compositions using the closest integral values for the number of residues of each amino acid. Since molecular weight determination by the method of partial substitution[3] on beef insulin has indicated a value in the range of 6,000, these minimum values were used as a basis for the numbers of residues of the six amino acids in Table I. All of the other amino acids are also present in approximately molar ratios when these molecular weights are used. Recent studies[4] by physical methods, however, have indicated a value for the molecular weight in the range of 12,000.

TABLE I

Amino acid	Beef: Amino acid residue per 100 g. protein, g.	Beef: Residues	Pork: Amino acid residue per 100 g. protein, g.	Pork: Residues	Sheep: Amino acid residue per 100 g. protein, g.	Sheep: Residues
Serine[a]	4.38	2.89	4.21	2.79	3.16	2.07
Threonine[a]	1.71	0.97	3.10	1.77	1.71	0.96
Glycine	3.92	3.94	3.89	3.94	4.70	4.70
Alanine	3.60	2.91	2.67	2.17	3.72	2.99
Valine[b]	8.10	4.68	6.31	3.68	8.34	4.80
Isoleucine[b]	1.30	0.66	3.02	1.54	1.36	0.69

[a] Corrected for decomposition during hydrolysis. [b] In the case of beef insulin the amounts of valine and isoleucine increased to molar proportions when the hydrolysis periods were increased to 48 hours and 96 hours, respectively.

Sanger[5] has obtained evidence by paper chromatography that four of the amino acids in Table I may be present in different amounts in beef, pork and sheep insulins. Lens and Evertzen[6] supported his conclusions. Other than these results, all studies based on physical methods, physiological activity and immunological specificity[7] have not shown species differences in purified insulins. Therefore the quantitative differences reported here must result in very slight physical and physiological differences. Species differences are well known to occur in the larger proteins. The results reported here suggest that similar species differences will be found in the smaller protein or peptide hormones.

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THE ACTION OF CARBOXYPEPTIDASE ON OVALBUMIN

Sir:

It has been shown by Desnuelle and Casal[1] and also by Porter[2] that the ovalbumin molecule contains no N-terminal amino acid residues according to the Sanger dinitrofluorobenzene (DNFB) method.[3] This finding might be accounted for in several ways: (I) end-to-end cyclization of the ovalbumin molecule; (II) internal cyclization of the N-terminal portion of the molecule; (III) masking of the α-amino groups due to combination with the non-protein moiety of the molecule (carbohydrate[4] or phosphate[5]); (IV) steric hindrance limiting the reactivity of free α-amino groups. If (I) were correct it should not be possible to demonstrate the presence of any free C-terminal residues. However, the present experiments in which the substrate specificity of carboxypeptidase[6-10] has been utilized indicate the presence of alanine as a C-terminal residue in ovalbumin.

A 3% solution of 4 times recrystallized ovalbumin was incubated at *p*H 7.4 with a suspension of commercial crystalline carboxypeptidase (Worthington) at a concentration of 0.08–0.15 mg. per ml. In order to rule out contaminating proteinase activities the experiments were repeated after three additional crystallizations of the enzyme.[11,12] No difference in results was noted. Aliquots of the reaction mixture were removed at intervals and adjusted to the isoelectric point of ovalbumin (*p*H 4.7) by the addition of 0.01 *N* HCl. Four volumes of absolute ethanol were then added and the alcoholic suspension was boiled for 5–10 min. The coagulated protein was removed by centrif-

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ugation and the supernatant, after being concentrated on the steam-bath, was subjected to paper chromatography for identification of the amino acids released. In most cases two-dimensional chromatography was used with 80% phenol–water as one solvent system and 70% propanol–water as the other. The papers were sprayed with 0.3% ninhydrin in water-saturated *n*-butanol and heated at 105° for 5 min. Controls of enzyme alone and of ovalbumin alone incubated under these experimental conditions were completely negative.

After 30 min. of incubation at 37° the only amino acid that could be demonstrated was alanine. After 60 min. of incubation only alanine could be detected immediately on heating of the paper. However, 24 hr. later very faint traces of several other amino acids became visible. After 120 min. of incubation the yield of alanine as estimated by the spot comparison method[13] was about 20% of the theoretical value calculated on the basis of one mole of alanine per mole of ovalbumin. Because the method of protein coagulation used here probably causes significant losses of free amino acids, this yield can only be taken as a minimum value. At 120 min. five other amino acids could be definitely identified, although present in much smaller amounts than the alanine. According to their R_F values in several solvent systems these were: valine, aspartic acid, glycine, glutamic acid and leucine (or isoleucine).

Subsequent incubations were carried out at 25° in an attempt to slow the reaction and establish, if possible, the sequence of appearance of these extra residues. Again at 25° the only amino acid released in short incubations (1 hr.) was alanine. Longer incubations (5 hr.) yielded larger amounts of alanine together with small amounts of the same five residues mentioned above. In one experiment the only amino acid that could be detected in addition to alanine was aspartic acid.

Since very little detailed information is available concerning the action of carboxypeptidase on intact proteins it was necessary to consider the possibility that the enzyme might be splitting open a cyclic molecule and then removing the C-terminal residue thus made available. If this were the mechanism an N-terminal residue should simultaneously become available (unless, of course, the non-protein moiety formed the bridge between N-terminal and C-terminal residues). The Sanger DNFB method[3] was applied both to native ovalbumin and to carboxypeptidase treated ovalbumin. No N-terminal residue could be demonstrated in either case.

It is concluded that alanine, at least, is a C-terminal residue in ovalbumin. The absence of free α-amino groups,[1,2] which has been confirmed here, is not due to end-to-end cyclization of the molecule but probably due to masking by the non-protein moiety.

It will be noted that the amino acids removed from ovalbumin by carboxypeptidase in the longer incubations are without exception to be found as component amino acids in the small peptides removed from ovalbumin by the *B. subtilis* enzyme of Linderstrøm-Lang and Ottesen.[14,15] While this may be coincidental it suggests very strongly that the peptides cleaved from ovalbumin when it is converted to plakalbumin occupy a C-terminal position in the intact molecule. In support of this hypothesis we have observed that the action of carboxypeptidase on plakalbumin no longer yields alanine predominantly as it does in the case of ovalbumin. Kinetic experiments will be necessary, however, to explore the possibility that one or more of the 5 amino acids appearing subsequent to alanine may also be C-terminal.

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THE VERATRINE ALKALOIDS. XXXIV. THE TRANSFORMATION OF ISORUBIJERVINE TO SOLANIDINE

Sir:

Studies on the tertiary veratrine bases indicate that all those thus far studied are members of a closely related group of hexacyclic substances which includes the potato base solanidine. All possess formulations of 27 carbon atoms and when subjected to selenium dehydrogenation, furnish a characteristic basic degradation product, 2-ethyl-5-methylpyridine.[1–3]

Previous studies on isorubijervine, $C_{27}H_{43}NO_2$ (*Veratrum album*[1,4] and *Veratrum viride*[5]) have shown that this alkaloid possesses a 3(β)-hydroxy-Δ^5-stenol character and the data at hand suggested that it, like rubijervine,[6] may be a hydroxy-solanidine.[7] Recently we have demonstrated this by the direct conversion of isorubijervine to solanidine by a method which appears to avoid any ambiguous stereochemical inversion.

Treatment of isorubijervine [m.p., 236–238°; $[\alpha]^{29}D$ +9.2° (*c* 1.1 in 95% EtOH)], in pyridine with *p*-toluenesulfonyl chloride preferentially yielded a primary mono tosyl derivative (II), m.p. 270–273° dec.; $[\alpha]^{30}D$ −36° (*c* 1.5 in abs. EtOH); *Anal.* Calcd. for $C_{34}H_{49}NO_4S$: C, 71.92; H, 8.70; S, 5.65. Found: C, 71.95; H, 8.58; S, 5.53. II was further characterized by oxidation with aluminum *t*-butoxide to the corresponding Δ^4-3-ketone (III), m.p. 316–319° dec.; *Anal.* Calcd. for $C_{34}H_{47}NO_4S$: C, 72.17; H, 8.37; S, 5.67. Found: C, 71.92; H, 8.37; S, 5.52. Oxime, m.p. 302–304° dec.; *Anal.* Calcd. for $C_{34}H_{48}N_2O_4S$: C, 70.31; H, 8.33; N, 4.82. Found: C, 70.10; H, 8.38; N, 4.82.

Treatment of II with sodium iodide in diethyl

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