procedure I it was therefore anticipated that the amount of radioactivity bound by experimental strips would exceed that in control strips by a similar amount. The much smaller difference observed (27 dpm/mg dry weight of tissue) is probably due to the fact that approximately 20% of the adrenaline taken up by the experimental strips is not removed by washing in our experiments* and prevents the subsequent uptake of ^{14}C-dibenamine hydrochloride. A study of the distribution of radioactivity in the lipid components of the lipid extracts (Table 3) confirmed those previously obtained.[3] In view of the unsuspected complications with labeling procedure I, it is planned to use labeling procedure II in our further studies.

It is necessary to point out, however, that even with procedure II, complications arise in the interpretation of our results. The major complication is the fact that adrenaline undoubtedly masks sites of dibenamine hydrochloride uptake other than the α-adrenergic receptors.

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Department of Pharmacology,
University of Alberta,
Edmonton, Alberta, Canada

M. S. YONG
G. S. MARKS

* M. S. Yong and G. S. Marks, unpublished observations.

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Amino acid composition of neurohypophysial secretory granules and Van Dyke protein*

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THE POSTERIOR pituitary is now known to secrete two major hormones, the octapeptides vasopressin and oxytocin. However, some early attempts at extracting the active principle from the gland resulted in the isolation of what appeared to be a single substance possessing both oxytocic and vasopressor activity.[1] Rosenfeld[2] submitted posterior lobe press juice to ultracentrifugation and found both hormone activities associated with material that sedimented as a single species. Van Dyke *et al.*[3] isolated a proteinaceous substance that was soluble in dilute acid and contained high activity of both hormones in a 1:1 ratio. The solubility, sedimentation, and electrophoretic properties of this material indicated that it was a single protein with a molecular weight of approximately 30,000. The Van Dyke protein was later shown to be a complex consisting of the two peptide hormones in non-covalent association with a protein component[4] subsequently named "neurophysin."[5] It was postulated

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that neurophysin is an inert carrier protein to which the hormones are bound and that the complex is stored in the neurosecretory granules (NSG) of neurohypophysial cells.

The NSG are membrane-bound vesicles about 100–300 mμ in diameter, although their size varies among species.[6] LaBella *et al.*[7] found about 20 units of each of the peptide hormones per mg of purified NSG protein, values very similar to those reported for the Van Dyke protein.[3, 4] Furthermore, the distribution of neurophysin in centrifugal fractions was reported to parallel the distribution of both vasopressin and oxytocin.[8] It appears that the NSG are packets of neurophysin in which the hormones are bound.

The present investigation was aimed at affording additional verification that the Van Dyke protein and the NSG protein are identical, by examining the amino acid composition of both preparations. The results of this work do, in fact, support the assumption that they are identical.

Neurosecretory granules were purified as previously described,[7] except that the density gradient was modified so that the NSG sedimented to the bottom of the tube, rather than at some intermediate position. With this modification, the NSG could be recovered by decanting the overlying suspension, thereby eliminating the necessity of fractionating the contents or slicing the tube in order to obtain the desired particle fraction. A density gradient was prepared by layering 10 ml of 22·5% sucrose onto 10 ml of 45% sucrose in 30-ml cellulose nitrate tubes and permitting them to stand at 4° for 2 hr. The suspension of the 48,000-*g* fraction was layered over the gradient, 5 ml per tube, and centrifuged at 64,000 *g* for 2 hr in a Spinco model L ultracentrifuge with an SW 25 rotor. Figure 1 shows the distribution of particles after equilibrium centrifugation. Three bands (A, B, and C) and a pellet (D) could

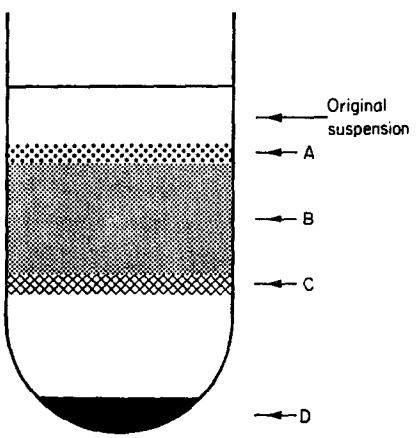

FIG. 1. Density-gradient centrifugation of the 48,000-*g* fraction. Three particle bands (A, B, C) and the NSG pellet (D) were obtained. The pellet was recovered by decanting the overlying mixture.

be distinguished. The pellet was recovered by decanting the supernatant and washed by suspending it in 10% sucrose and centrifuging at 100,000 *g* for 30 min.

The Van Dyke protein was prepared from bovine posterior pituitary glands by the method of Van Dyke *et al.*[3]

For amino acid determinations NSG or Van Dyke protein was hydrolyzed for 48 hr in 5 N HCl at 110° under nitrogen in sealed glass vials. Amino acid separation was carried out on a Technicon instrument, with B resin in a column 0·63 × 75 cm and a 6-hr system. The amino acid values are not corrected for destruction during hydrolysis. Cystine plus cysteine were determined as cysteic acid by the performic acid oxidation method of Moore.[9]

Vasopressin was assayed by a modification[10, 11] of the rat blood pressure method of Landgrebe *et al.*[12]

The amino acid composition of three preparations of NSG and four preparations of Van Dyke protein are presented in Table 1. For either NSG or Van Dyke protein, determinations on preparations from different batches of bovine glands agree quite well. The only significant difference between NSG and Van Dyke protein is found for glycine, which is higher in the latter. Slightly lower values for most of the other amino acids are found for the Van Dyke protein as compared to NSG. The considerably higher glycine content of the Van Dyke preparation would, by necessity, cause a corresponding decrease in the relative proportion of the other amino acids of the total.

TABLE 1. AMINO ACID COMPOSITION OF NEUROSECRETORY GRANULES AND VAN DYKE PROTEIN

Prep.	NSG				Van Dyke Protein					NSG/Van Dyke
	1	2	3	Avg	4	5	6	7	Avg.	
	Residues per 1000 total residues*									
Asp	108	100	98	102	99	71	78	103	88	1·16
Thr	44	39	41	41	31	27	57	50	41	1·00
Ser	68	66	56	63	38	47	60	58	51	1·24
Glu	133	145	117	132	117	151	164	126	140	0·94
Pro	68	70	91	76	87	118	99	61	91	0·84
Gly	115	122	107	115	152	208	184	135	170	0·68
Ala	96	94	96	95	88	72	64	88	78	1·22
Val	64	64	59	62	58	46	63	59	57	1·09
Ileu	39	37	38	38	38	35	33	44	38	1·00
Leu	86	96	90	91	83	72	72	88	80	1·14
Tyr	14	9	29	17	18	16	14	26	19	0·89
Phe	36	39	41	39	37	34	36	40	37	1·05
Lys	54	51	50	52	49	35	27	50	40	1·30
His	20	18	16	18	16	9	5	15	11	1·64
Arg	54	54	72	60	59	65	66	55	61	0·98
Cys (1/2)	4·2†		3·7†			16·5†		5·2†		
Vasopressin (U/mg)	19·4	22·7	16·3		13·4	14·9	17·4			

* Excluding cystine and methionine.
† Per cent of total nitrogen recovered.

Cystine (cysteine + cystine) appears to be lower in NSG than in Van Dyke protein. In one determination the 16·5 per cent cystine content of the Van Dyke protein is very close to that reported by Block and Van Dyke,[13] whereas the second value on another preparation was 5·2 per cent; both values are higher than those found for NSG. It should be pointed out that the single amino acid estimation by Block and Van Dyke was carried out by quantitative paper chromatography. Methionine, which tends to be destroyed by acid hydrolysis, was not determined separately, since methionine sulfone produced during performic acid oxidation in the determination of cystine of this amino acid was not resolved. However, it could be determined that small quantities were present in one or two preparations of both NSG and Van Dyke protein. Methionine is neither consistently present nor is it found in any more than trace amounts when present.

The hormone contents are in the range previously found by us for NSG[7] and for Van Dyke protein by others.[3, 5] We consistently attain higher hormone activities for NSG than for Van Dyke protein. Dialysis of a preparation of Van Dyke protein against acetic acid, a procedure that completely removes hormonal activity, failed to alter the amino acid composition noticeably, the differences being within the range of variability of our analytical method.

The amino acid composition of the NSG was found to be very similar to that of the Van Dyke protein, thereby indicating the correctness of the assumption that the latter does, in fact, represent the neurosecretory material contained within the NSG. The assumption was supported in another way by Ginsburg,[8] who purified neurophysin from subcellular fractions of posterior pituitary glands and found its distribution parallel to that of the peptide hormones. There is probably some degree of

contamination in both the NSG and Van Dyke protein; for example, our NSG preparations contain, at best, traces of enzyme activities known to be associated with other types of particles. It is interesting to note that, among the slight differences in amino acid content between the two preparations, glycine cystine, and glutamic acid are higher in the Van Dyke protein than in the NSG. These three amino acids are constituents of glutathione, which is abundant in the posterior pituitary gland (unpublished observations). The Van Dyke protein preparation (no. 5 in Table 1) that contained 16·5% cystine, the highest value found for this amino acid, also contained relatively high values for glycine and glutamic acid. Glutathione is possibly co-extracted from the gland during the purification of the Van Dyke protein. We hope to examine this possibility. If contaminating protein is present in either of the preparations, it probably represents only a minor proportion of the total protein, or one would not expect such close agreement in the amino acid composition of the NSG and the Van Dyke proteins. A further similarity that indicates the synonymity of the two preparations resides in their hormonal activity. It would appear extremely fortuitous for two dissimilar proteins to bind the peptide hormones identically.

The work of Ginsburg[14] and Hope[15] and their co-workers has shown that neurophysin preparations are composed of multiple protein components when subjected to chromatography, and Ginsburg and Ireland[14] have shown also that these different components have widely varying capacities to bind vasopressin or oxytocin. It is possible that the carrier protein contained within any single NSG and to which the peptide hormones are bound is, in fact, heterogeneous. On the other hand, work in our laboratory[7] and in others[16, 17] has indicated that some degree of separation of the two hormone activities can be achieved during centrifugation of subcellular particles. Although particles containing one hormone exclusively have not yet been isolated, there is considerable evidence to suggest that neurosecretory granules specific for one or the other of the hormones do exist in the neurohypophysis. Thus, multiple components in neurophysin or NSG preparations might represent structurally distinct protein species, only one of which is localized in any given neurosecretory granule.

Department of Pharmacology and Therapeutics,
University of Manitoba Faculty of Medicine,
Winnipeg,
Manitoba,
Canada

FRANK S. LABELLA†
STANLEY VIVIAN
ELLIOT BINDLER‡

† This work was done during the tenure of an Established Investigatorship of the American Heart Association, Medical Research Associate, Medical Research Council of Canada.

‡ Present address: Department of Psychiatry and Neurology, New York University Medical Center, 550 First Ave., New York, N.Y. 10016.

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Inhibition of succinoxidase activity in normal sarcosomes by sarcosomes from Antimycin-A perfused guinea pig heart

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ANTIMYCIN A is a known inhibitor of the electron transport pathway and would appear to act either on an unknown component between cytochromes *b* and *c* or directly with cytochrome *b* to prevent its oxidation.[1, 2] In previous investigations[4] it was found that perfusion of guinea pig heart with 0·2 μg Antimycin A per ml of Locke–Ringer solution resulted in: (1) early ino- and chrono-tropic negative effects; (2) continued beating of the heart even after 20 min perfusion with Antimycin A, and (3) persistence of negative ino- and chrono-tropism even after perfusing with a medium devoid of antibiotic. The latter behaviour is contrary to that observed for other inhibitors of the oxidative pathway.[3] Since Reif and Potter[5] observed that Antimycin A attached itself firmly to mitochondria for a period of time, it was decided to investigate the behaviour of the sarcosomes of the perfused heart, using succinoxidase activity as a marker of the oxidative pathway, in order to further determine the nature of the effect of Antimycin A on guinea pig heart.

Guinea pig hearts were perfused with 0·2 μg of Antimycin A per ml of Locke–Ringer solution for (a) 5 min; (b) 10 min, and (c) 10 min followed by perfusion for 20 min with Locke–Ringer solution devoid of the antibiotic. Sarcosomes were isolated by the method of Hogeboom[6] from both normal guinea pig hearts and from hearts which had been perfused as described above. In order to ensure that no free Antimycin A remained in the medium, this preparation was washed repeatedly. Sarcosomal protein was determined by the method of Greenberg[7] and succinoxidase activity by the polarographic technique employing the Clark electrode.[1]

Succinoxidase activity in sarcosomes from hearts perfused for 5 min with Antimycin A showed only 10 per cent activity, whilst that from hearts perfused for 10 min showed complete inhibition. Succinoxidase activity was inhibited totally in sarcosomes from hearts perfused for 10 min with Antimycin A followed by perfusion for 20 min with Locke–Ringer solution. These data are in agreement with those of Reif and Potter,[5] and substantiate their suggestion of a binding of Antimycin A by the sarcosomes in the intact heart with the subsequent depression of succinoxidase activity.

The addition of Antimycin A *in vitro* to a normal sarcosomal preparation, in a final concentration of 0·29 μg/mg sarcosomal protein, also inhibited succinoxidase activity (Fig. 1, trace I), in agreement with the data of Ackerman and Potter[8] and of Ahmed *et al.*[9] This was the minimal amount of Antimycin A sufficient to inhibit completely succinoxidase activity under these experimental conditions. If an aliquot of normal sarcosomes was added to an aliquot of Antimycin A-treated sarcosomes, the succinoxidase activity of the combined sarcosome preparations was equivalent to that of the expected activity of the added aliquot (Fig. 1, trace I).

In contrast, if an aliquot of the sarcosomes from Antimycin A-perfused hearts was added to an aliquot of normal sarcosomes, there was an immediate, total inhibition of succinoxidase activity (Fig. 1, trace II). There was a similar total inhibition if an aliquot of normal sarcosomes was added to an aliquot of sarcosomes from Antimycin A-perfused hearts.