Physical Release of Acetylcholine from the Thoracic Nerve Cord of *Periplaneta americana* L.

Recently, suggestions have been made that acetylcholine in nerve tissue is associated with structural entities termed 'vesicles'[1]. The ideas are compatible with a quantum concept of nerve transmission[2]. The evidence that acetylcholine is released from mammalian nerve tissue by physical means[3] lends further support to the structural inclusion of acetylcholine within the nerve cell and casts doubt on the hypothesis that it is held in chemical combination. The following results on the release of acetylcholine from cockroach thoracic cord by osmotic pressure and homogenization makes the existence of a chemical combination in insects doubtful.

Table 1. Effect of Osmotic Pressure on Amounts of Acetylcholine in Supernatant and Residues of Homogenates of Cockroach Thoracic Cord

Extraction molarity with medium homogenization (17 sec.)	Acetylcholine			
	Total (μgm.) (15 cords)	Supernatant (μgm.)	Residue (μgm.)	Supernatant (per cent)
Distilled water	5·75	4·75	1·0	82·6
0·244 *M* Ringer	4·57	3·18	1·39	69·5
0·314 *M* Ringer	4·68	2·85	1·83	60·8

The results in Table 1 show the effect of extraction media of different osmotic pressures on the amounts of acetylcholine recovered from supernatants and residues of homogenates of thoracic cords of cockroaches. The cords were homogenized for 17 sec. in ice-cold medium and centrifuged at 50,000*g* for one hour. Assays for acetylcholine were carried out on frog rectus abdominus muscle, and the values are expressed as chloride. It is clear that both the supernatant and residue values for acetylcholine depend upon the tonicity of the extraction medium. The relatively hypertonic medium, 0·314 *M*, which gave the lowest supernatant value, has the equivalent osmotic pressure of Hoyle's insect Ringer. Furthermore, desheathed cockroach ganglia do not swell at this osmotic pressure. Having established an extraction medium apparently suitable to this insect nerve tissue a combination of homogenization and osmotic pressure was employed to release acetylcholine. The results are shown in Table 2. It is striking that the hypotonic medium, distilled water, released a high proportion of acetylcholine into the supernatant, whatever the severity of homogenization. In the instance of the hypertonic medium more acetylcholine was released into the supernatant with increasingly severe homogenization, until the value for acetylcholine in the supernatant approximated that of distilled water. It should be noted that in either medium it was not possible to obtain all the acetylcholine in the supernatant at severe homogenization.

It is concluded from these results that acetylcholine in cockroach thoracic cord can be released by both osmotic pressure and mechanical disruption. The ease with which acetylcholine can be released in insects supports the suggestion that in insects it can be released more easily than in mammals (Pal, R., unpublished). The evidence favours the hypothesis that acetylcholine is not in chemical combination in insects but located in the nerve cell within a 'structural compartment'.

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Method of isolating the β_1-Metal-combining Globulin from Human Blood Plasma

Surgenor, Koechlin and Strong[1,2] have described the isolation of the β_1-metal-combining globulin (siderophilin, transferrin) from Cohn's plasma fraction IV–4 by alcohol fractionation. The crystallization, described by Koechlin[2], could not easily be reproduced by other investigators. Improved conditions for the purification and the crystallization starting from fraction IV–7 have recently been published by Inman[3]. Already the preparation of fraction IV–7 requires many steps and much time, which may be one of the reasons why only very few clinical experiments with this iron-transmitting globulin have been reported.

We have found that an electrophoretically almost pure preparation of the metal-binding globulin can be obtained by a much shorter procedure using rivanol (2-ethoxy-6,9-diaminacridinelactate).

Hořejši and Smetana[4] have shown recently that rivanol precipitates from serum or plasma at *p*H 7·3 almost all the proteins except the γ-globulin (2 parts of plasma + 7 parts of 0·4 per cent rivanol solution at room temperature). After adsorption of the rivanol by charcoal, the γ-globulin can be precipitated from the supernatant in very good yield under conventional conditions.

We have found that the supernatant of the rivanol precipitation contains significant amounts of albumin and a highly soluble β-globulin besides γ-globulin. The higher the *p*H during the rivanol precipitation, the smaller are the amounts of albumin found in the supernatant. Between *p*H 8 and 10 albumin is precipitated almost quantitatively, while the supernatant still contains γ-globulin and the soluble

Table 2. Effect of Homogenization and Osmotic Pressure on Amounts of Acetylcholine in Supernatants and Residues of Homogenates of Cockroach Thoracic Cord

Degree of homogenization	Acetylcholine							
	Distilled water				Insect Ringer 0·314 *M*			
	Total (μgm.) (15 cords)	Supernatant (μgm.)	Residue (μgm.)	Supernatant (per cent)	Total (μgm.) (15 cords)	Supernatant (μgm.)	Residue (μgm.)	Supernatant (per cent)
Light (5 sec.)	5·85	4·35	1·5	74·3	5·43	2·9	2·53	53·4
Medium (17 sec.)	5·75	4·75	1·0	82·6	4·68	2·85	1·83	60·8
Waring blendor (1 min. at 16,000 r.p.m.)	5·5	4·5	1·0	81·8	5·05	3·3	1·75	65·3
Medium + Waring blendor	4·65	4·28	0·37	92·0	4·52	3·86	0·66	85·4