vesicles (Figure 3), resembling the synaptic vesicles of mature synapses[11]. These vesicles seem to be best preserved in material postfixed with potassium permanganate. In some cases, isolated vesicles of 1000–1400 A in diameter, with a central dense core, can be seen along cell processes or in cell somas.

Neuroblasts found in aggregates formed by T or NR cells have the same morphological characteristics, although the latter show a peculiar spatial organization which has been described by SHEFFIELD and MOSCONA[8]. Besides occasional clusters of synaptic-like vesicles, no other synaptic elements could be observed in their processes.

'Combined' aggregates. 'Combined' OL+NR and OL+T aggregates do not show any striking ultrastructural differences when compared with 'pure' OL, NR or T ones. Although both types of 'combined' cultures apparently have a greater number of cell processes, morphological synaptic differentiation seems to be at the same stage as in 'pure' aggregates. ADLER and TEITELMAN[12] observed that, after 8 days in culture , the activity of the enzyme choline-acetyltransferase, which is responsible for the synthesis of acetylcholine, is higher in 'combined' OL+NR aggregates than in 'pure' OL or NR ones. It could seem surprising that no difference in synapse formation is found when the ultrastructure of the same aggregates is compared. On the basis that biochemical and structural aspects of differentiation must not necessarily be synchronical (see also ref.[13]), we are now studying the same type of aggregates kept in culture for longer periods.

In OL+LB 'combined' aggregates, both types of cells show a peculiar sorting-out behavior (Figure 4). After 8 days in culture, LB cells form a central core of stellated cells immersed in an amorphous extracellular substance and surrounded by a shell of elongated cells. OL cells appear forming clusters adherent to this outer shell. This tendency of reconstructed neural tissue to cover only partially other tissues in 'combined' cultures has been described by STEINBERG[14]. In the neural region of these OL+LB aggregates, there seems to be a greater number of cell processes and, moreover, synapse formation seems to be more advanced than in 'pure' OL aggregates. Many cell processes show clusters of synaptic-like vesicles of the clear type and/or larger vesicles (800–1500 A) containing a central dense core. In these 'combined' aggregates, the clusters of vesicles often appear associated with membrane specializations which suggest the existence of a synaptic complex (Figures 5 and 6).

Other authors have shown that embryonic neural cells in reaggregation cultures are able to attain functional synapse differentiation[9]. The length of time required for the appearance of synaptic structures in vitro depends on the age and type of donor tissue[3,8,13,15–17]. Seven-day-old optic lobe cells, which at this age have not formed synaptic structure in vivo[18], are able to form synapse-like structures after only 8 days in culture in combination with limb bud cells, although they fail to do so in 'pure' cultures, or in combination whit telencephalon or neural retina cells. Limb bud cells, therefore, seem to provide some factor which stimulates synapse formation by optic lobe cells[19,20].

Resumen. Se describe la diferenciación ultraestructural alcanzada en cultivos de reagregación por células neurales embrionarias despues de 8 días in vitro. Se estudiaron agregados «puros» formados por células de lóbulo óptico, retina neural o telencéfalo, y cultivos «combinados» de lóbulo óptico + retina neural (OL + NR), lóbulo óptico + telencéfalo (OL + T), y lóbulo óptico + esbozo de miembro (OL + LB). En los cultivos «puros» las células poseen características de neuroblastos, pero el proceso de sinaptogénesis está poco desarrollado. No se encuentran diferencias significativas entre la diferenciación alcanzada por los cultivos «puros» y los «combinados» OL + NR y OL + T. Por el contrario, en los «combinados» OL + LB la sinaptogénesis está mucho más avanzada y se encuentran prolongaciones sinápticas semejantes a las del adulto.

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The Allergenic Cross-Reactivity of Antisera made Against Different Molecular Weights of a Homopolymer, Dextran

Dextran, a homopolymer consisting of 96% α-1,6 linked glucose units can be obtained in fractions ranging in molecular weight from 1400 to 1.9×10^6[1]. The homopolymer may exist in preferred configurations[2]. Studies utilizing this homopolymer have demonstrated that the valency of antibody, both of the IgG and IgM class, vary with the size of the dextran[3]. Bivalent haptens can elicit the passive cutaneous anaphylaxis reaction and precipitate with antibody whereas univalent haptens cannot. RICHTER[4] has shown that a passive anaphylactic reaction (PCA) can occur if the antiserum is of sufficient strength and with dextran homopolymers of molecular weight as low as 3600. This study was undertaken to determine the degree of cross-reactivity between antibody made against various sized dextran fractions and to determine the lower limit molecular weight which will elicite an allergic reaction.

Materials and methods. New Zealand white rabbits were immunized with dextran molecular weight fractions 1400, 2950, 4875, 10,000, 110,000 and 1.9×10^6 coupled to

The relationship between the molecular weight of dextran, serving as ligand, and the passive cutaneous anaphylaxis (PCA) reactions obtained with antisera made against each dextran fraction

Antibody to dextran	Ligand size					
Fraction	1400	2950	4875	10,000	110,000	1.9×10^6
1400	0	±	2+	2+	2+	2+
2950	0	1+	2+	2+	3+	2+
4875	0	1+	3+	4+	4+	2+
10,000	0	1+	2+	4+	4+	2+
110,000	0	0	1+	4+	4+	4+
1.9×10^6	0	0	0	2+	3+	4+

The degree of PCA is measured for each fraction, as compared to a nonsensitized panel of guinea-pigs, from no detectable PCA (0) to maximum PCA (4+).

human IgG utilizing cyanuric chloride. Dextran fractions of molecular weight 10,000 and above were commercially obtained (Pharmacia Co., Piscataway, New Jersey); dextran fractions of lower molecular weights were obtained by acid hydrolysis and ultrafiltration. All fractions were physically and chemically analyzed[5]. Molecular weight was determined by equilibrium sedimentation measurements made in a Beckman model E ultracentrifuge[5] and molecular number was determined by the endgroup method of ISBELL[6].

Male albino guinea-pigs weighing between 250 and 300 g were sensitized with 1.0 ml of antiserum 24 h prior to their receiving antigenic challenge. The animals were challenged with 1.0 mg of dextran in 2.5 ml of Evans blue dye i.v. 15 min post-challenge the animals were sacrificed and the diameter of dye permeation on the under surface of the skin measured. 6 animals were utilized for each fraction studied.

Results and discussion. High-titer antiserum was raised to all dextran fractions. All diameters of Evans blue dye permeation were compared to a standard, nonsensitized panel of guinea pigs. Each value (see Table) was the average of 6 guinea-pigs, the average being graded 1+ to 4+ with 0 not being significantly different from the unsensitized panel. 4+ represented the zone diameter of greatest permeation for a given dextran fraction. This system was choosen to obviate the different diffusion coefficients of the dextran fractions and make results between fractions comparable.

Passive cutaneous anaphylaxis could be elicited reproducibly by dextran fractions of molecular weight 4875 and higher and with molecular weight 2950 with 50% of our antisera. Challenge with molecular weight 2950 gave 1+ reactions with antisera against dextran fractions molecular weight 2950, 4875 and 10,000 while negative reactions with antisera against dextran fractions 1400, 110,000 and 1.9×10^6. Molecular weight 2950 was, in this series, the lowest molecular weight hapten able to elicit PCA.

It appears, as shown in the Table that a non-homogeneity exisst, to some extent, concerning the cross-reactivity of disparate molecular weight fractions with antisera raised to dextran fractions of varying sizes. Antisera raised to dextran fractions in the higher molecular weight range elicite the PCA reaction to a greater degree with high molecular weight dextran than with low molecular weight dextran. Likewise, antisera raised against low molecular weight dextran elicited the PCA reaction to a greater degree with low molecular weight dextran fractions than high molecular weight dextran fractions.

Since the dextran molecule is neutral[7], consists of only one type of antigenic determinant[8,9] and varies only with respect to size in its physical-chemical properties, any changes in immunological reactivity amongst dextran fractions must be due to either changes in the valency of the antibody in respect to dextran or steric configurational changes occurring between different sized dextran fractions. Since immunological specificity lies in the α-1,6 region heterogenity of antibody production is effectively ruled out. These differences may be due to a combination of the steric hindrance of antibody combining sites by large molecules and the greater degree of spacial specifity that could be present among the larger fractions. KABAT et al.[2] has reported that the dextran molecule may exist in preferred configurations. There is quantitative evidence that the valency of antibody is not the same for small and large dextran molecules. Dextran molecular weight 1.9×10^6 lowers the valency of IgG from 2 to 1.1 and IgM from 10 to 2.3[3]. This inherent difference of binding abilities of large and small molecular weight dextran fractions would have nothing to do with the antibody molecule per se. Therefore, inately, extremely large homopolymers may function less well in serological reactions due to their lowering of the valency of the antibody. Large-size molecules could be expected to present their immunogenic groups in spacial configurations with which the smaller groups are incapable due to the opportunities offered by size.

Zusammenfassung. Nachweis einer Kreuzaktivität zwischen Antikörpern gegen Dextran von verschiedenen Molekulargewichten und Bestimmung der niedrigsten Fraktionen, die noch eine allergische Reaktion auszulösen vermag.

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