likewise be cholinergic. Thus the thalamus, which we have shown to contain ChE within the cell bodies of neurones belonging to the anterior group of nuclei and in nerve terminals derived from the dorsal tegmental pathway, has been found in dog to be rich in acetylcholine[19] and in cholinacetylase[20]. It has been suggested that ChE may act at sites of constant ACh production to prevent the concentration of ACh from passing beyond a certain level[21]: since ChE does not exhibit the phenomenon of substrate inhibition with respect to acetylcholine, it continues to act at concentrations of the latter which would inhibit AcChE. Another possibility is that the *in vivo* action of ChE is on higher choline or related esters. Koelle[22] has argued that the ChE which is present in large quantities in addition to AcChE in Auerbach's plexus may be concerned with the hydrolysis of such esters acting as local hormones or transmitting agents in the gut. These esters might act directly on the receptor or might have a potentiating effect, just as butyrylcholine will not only cause contraction of frog's rectus but will also sensitize the muscle to the action of acetylcholine[23]. It is not certain, however, that higher cholinesters occur in normal brain[24], other than γ-aminobutyrylcholine, which may function as an inhibitory transmitter[25].

Other sites of acetylcholine activity where ChE is present in addition to AcChE include the motor end plates of rat diaphragm[26]. We have studied the cholinesterase content of motor end plates in the tongue musculature of rat, and our observations suggest that the proportion of ChE is greatest in those of the intrinsic muscles. It is noteworthy that these muscles are believed to be innervated from the ventral part of the hypoglossal nucleus[27], which we have found to contain intraneuronal ChE. The other location for ChE in the medullary portion of the floor of the fourth ventricle is in the cells which form the dorsal motor nucleus of the vagus. These neurones are generally held to be cholinergic, although the evidence, based mainly on the acetylcholine content of the cervical vagal trunk[19], is not conclusive since some at least of the cholinergic fibres in the cervical vagus are probably derived from the sympathetic system[28].

Some importance may be attached to the distributional pattern of intraneuronal ChE in the central nervous system. It is noticeable, for example, that a number of the sites in which this enzyme is concentrated, such as the interstitial nucleus of the hippocampal commissure, the dorsal and deep tegmental nuclei, and the anterior thalamic nuclei, are on projection pathways related to the hippocampal fornix system. Other nuclei containing intracellular ChE, for example, the nucleus reticularis tegmenti pontis[29] and the nucleus reticularis lateralis[30], are known to project on to the cerebellum. Green and Morin[31] found electrophysiological evidence, which they were not then able to explain, for a functional relationship between fornix pathways and the cerebellar cortex. The projection of ChE-containing neurones on to the midline and intralaminar thalamic nuclei must, of course, be considered in relation to the diffuse thalamic projection system of Morison and Dempsey[32] which originates from these nuclei. It is significant, therefore, that high-frequency repetitive stimulation of the midline and intralaminar nuclei produces electrocortical arousal[33], and that a similar effect can be achieved by intra-arterial injection of ChE inhibitor[34]. Certain of the midline and intralaminar nuclei have been shown to project to the corpus striatum[35], and the final link with the cortex responsible for bringing about the arousal reaction may be achieved through the corticopetal projections of the AChE-containing, presumed cholinergic cells which we have found in this region.

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RESEMBLANCES BETWEEN PASSIVE ANAPHYLACTIC SENSITIZATION AND TRANSMISSION OF PASSIVE IMMUNITY

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RECENT advances in knowledge of passive anaphylactic sensitization and of transmission of passive immunity from mother to young have disclosed certain similarities in these two processes which it would seem worthwhile to consider, since it is possible that too little attention has been paid by those engaged in the one field to the results obtained in the other.

Hartley[1] was the first to suspect a similarity between the transmission of passive immunity from mother to fœtus and passive anaphylactic sensitization in guinea pigs and

to suggest that both processes depended on the acceptability of the antibody molecules to the cells. He knew that guinea pigs could be readily sensitized by antitoxins prepared in guinea pigs, rabbits or man, but not by those prepared in horses, oxen, sheep, goats or pigs. He showed that whereas guinea pig antitoxin was transmitted readily from mother to fœtus, horse antitoxin was scarcely transmitted at all. Further, he showed[2] that diphtheria antitoxin prepared in a guinea pig, when pepsin refined, lost the capacity both for passive sensitization and for passive transmission, although retaining its antibody activity.

During the past ten years much additional evidence has been obtained which tends to justify Hartley's assumption. To these advances, increasing knowledge of the structure of the γ-globulin molecule has contributed notably. It is unfortunate, however, that whereas most of the work on anaphylaxis has been on the guinea pig, most of that on the transmission of passive immunity has been on the rabbit, rat and mouse. Parallel studies on both these phenomena in a single species are needed for precise comparison and they should be rewarding.

Anaphylactic sensitization of guinea pigs *in vivo* and of guinea pig tissues *in vitro* is a selective process in so far as γ-globulin is the only effective agent, and the γ-globulins of some species are more effective than those of others. Humphrey and Mota[3] found, for example, that guinea pig and rabbit antibodies are the most effective, monkey, dog and human antibodies somewhat less effective, and rat, horse, goat and fowl antibodies ineffective. Antibodies from oxen and sheep are ineffective also[1], as are human auto-immune antibodies[3] and those to pollen[4].

Immunity is transmitted before birth in the rabbit and guinea pig and mainly after birth in the rat and the mouse; the γ-globulin being transmitted preferentially to the other serum proteins in all these animals. Antitoxins prepared in various species are transmitted preferentially in the order rabbit > human > guinea pig > dog > horse > ox in the rabbit. Rabbit antitoxins are transmitted in quantities about 100 times those of bovine antitoxins[5]. Rabbit, rat, guinea pig and other rodent antibodies are transmitted readily from the gut to the circulation in the suckling rat, but hyperimmune agglutinins to *Salmonella pullorum* from these species are transmitted several times more readily than agglutinins to *Brucella abortus* prepared in the same species[6]. Antibodies prepared in ox, sheep and fowl are not transmitted[7,8]. Some antibodies are transmitted preferentially in the order mouse > rat > guinea pig > rabbit > ox in the mouse[9,10]. However, when hyperimmune rabbit anti-*Brucella* serum containing a high titre of complete agglutinins is fed to suckling rats and mice a considerable quantity of incomplete agglutinins, with little or no complete agglutinins, appears in the circulation. Thus there is a notable similarity between passive anaphylactic sensitization and passive transmission of immunity in that both processes are selective between immune globulins derived from different species as well as in some cases between different antibodies derived from the same species.

Hartley[2] was the first to observe that diphtheria antitoxin prepared in guinea pigs, when pepsin refined, lost both the properties of passive sensitization of guinea pig smooth muscle tissue *in vivo* and *in vitro*, and of passive transmission from the maternal to the fœtal circulations in this species, although retaining unimpaired its antibody activity. The rate of transmission across the rabbit fœtal membranes of homologous diphtheria antitoxin is much reduced by pepsin refining[11]. Pepsin refining reduces the size of the antitoxin molecule by about one-third without impairing its antibody activity but with loss of most of its antigenicity. This suggested that the part of the molecule destroyed by pepsin was that responsible for anaphylactic sensitization and for the rate of transmission across the fœtal membranes. This part of the molecule also appears to be that which carries most of the antigenic determinants which distinguish the γ-globulin of one species from that of another. The discovery at this time[12] that the parts of rabbit γ-globulin molecules lost by pepsin digestion could be recovered after digestion with crystalline papain and separated chromatographically in a crystallizable state opened up the way for testing this hypothesis. This technique yielded also two fractions with antibody activity, each of which consisted of molecular fragments of about one-half the size of the product of pepsin digestion. Each fraction had incomplete or monovalent antibody activity as distinct from the complete or divalent activity of pepsinized antitoxin. These fractions were called I and II and the antigenic fraction was called III. It has been shown since[13] that a molecule of rabbit γ_1-globulin is composed of two identical pieces of fraction I together with one piece of fraction III, and that the molecule of rabbit γ_2-globulin is similarly constructed of two pieces of fraction II and one of fraction III. It has been shown[14,15] that neither fraction I nor fraction II of rabbit antibody is able to sensitize the skin of the guinea pig either for direct or reversed passive cutaneous anaphylaxis, whereas fraction III is an effective sensitizing agent in reversed passive cutaneous anaphylaxis. It was concluded that whereas guinea pig cells could readily fix fraction III, they could not fix fractions I or II of rabbit γ-globulin. Fraction III of rabbit γ-globulin, isotopically labelled with iodine-131, is transmitted across the membranes to the fœtal circulation of the rabbit eleven times as readily as fraction I and six times as readily as fraction II[16]. Fraction III was transmitted at nearly the rate (0·7) of whole rabbit γ-globulin similarly labelled. This suggested that the region of the γ-globulin molecule which contains the 'recognition unit' for transmission by homologous cells is also that (fraction III) which contains most of the antigenic groups for heterologous cells. The close correspondence of the conclusions that have been drawn from the use of these molecular fragments in passive anaphylactic sensitization and in the transmission of passive immunity is obvious.

Non-immune serum can interfere with the passive sensitization of guinea pig tissues by antibodies[17]. This interference is due to the γ-globulin fraction of the serum, not to the other serum proteins[18]. It can be demonstrated *in vivo* in passive general anaphylaxis in both guinea pigs and mice[19], and in passive cutaneous anaphylaxis in guinea pigs[20]. It can be demonstrated *in vitro* in guinea pig tissues by the Schultz–Dale reaction of smooth muscle[3,21] by histamine release from chopped lung[22] and by mast cell degranulation[3]. The γ-globulins of different species vary in their capacity to inhibit passive sensitization in guinea pigs; for example, the effectiveness of γ-globulins to interfere with the sensitization by rabbit antibodies of guinea pig smooth muscle tissue decreases in the order rabbit > man > dog > guinea pig > rat > horse > ox > pig > fowl > goat[23]. For complete inhibition of sensitization the non-specific γ-globulin must be present in amounts considerably in excess of the antibody, thus for inhibition of passive cutaneous sensitization in the guinea pig by rabbit antibody a ratio of antibody/non-specific γ-globulin of 1 : 100 is required[21]. Fraction III of the papain hydrolysed rabbit γ-globulin is even more effective than the whole γ-globulin in inhibiting the sensitization of guinea pig tissues by rabbit antibody, whereas fraction I is entirely without effect[15].

Certain heterologous sera interfere with the transmission of antibodies from the gut to the circulation of suckling mice and rats when administered orally at the same time[24]. The transmission of both homologous and heterologous antibodies can be interfered with in this way, but only the sera of certain species interfere. Rat, mouse, hamster and sheep sera do not interfere with the transmission of antibodies in either the suckling mouse or suckling rat. Rabbit, guinea pig, human and bovine

sera are effective in producing interference in both rats and mice; all do so about equally in the rat, whereas in the mouse the order is rabbit > guinea pig or human > bovine serum. The γ-globulin fraction is at least as effective as whole serum and can account for the whole of the interference, whereas the albumin fraction has no effect. However, to inhibit transmission of immunity, the γ-globulin, whether in serum or separated, must be in considerable excess relative to the antibody globulin. Fraction III of papain hydrolysed rabbit γ-globulin is several times as effective as whole rabbit γ-globulin in interfering with the transmission of antibodies in mice, whereas fractions I and II are ineffective[25]. Interference has not been observed in the transmission of antibodies across the fœtal membranes in rabbits and no information is available concerning other species.

Thus, the phenomenon of interference has been observed both with passive anaphylactic sensitization in the guinea pig and with passive transmission of immunity in the rat and the mouse. The sera of certain species interfere, whereas those of others do not, both in anaphylaxis and in transmission. In both processes the whole effect is due to the γ-globulin fraction of the serum, and fraction III of papain hydrolysed rabbit γ-globulin is considerably more effective than the whole γ-globulin, whereas fraction I is without effect. The interfering γ-globulin must be in substantial excess over the immune γ-globulin for interference to approach completion in either process. Both in anaphylaxis and in transmission interference has been attributed to competition for specific receptors on, or in, the cells.

The resemblances mentioned strongly support the assumption of an essentially similar mechanism of cellular attachment in both sensitization and transmission. There are, however, important differences between the two processes. Perhaps the most obvious is that, whereas it is the mast cells which are involved in sensitization, it is the endoderm cells of either the fœtal yolk-sac or of the fœtal or neonatal gut which mediate transmission in rabbits and rodents, although the syncitiotrophoblast may be concerned in primates[26]. Secondly, whereas sensitization only involves the assumption of attachment to receptors on the external surfaces of the cells, transmission requires transport across the cells from one pole to the other and, hence, the assumption of internal receptors. All the available evidence, both physiological[27] and electronmicrographic[28,29], indicates that in the transmission of immunity the uptake of the γ-globulin by the cells is pinocytotic and, consequently, not selective. This suggests that the receptors are probably in the walls of the pinocytotic vesicles, although they may be carried from the external surface into the vesicles when they are formed. The excess protein in solution, absorbed pinocytotically and not transmitted, is certainly degraded so that absorption and transmission are not commensurate[27]. Finally, whereas sensitization does not necessarily require a mechanism of release of the γ-globulin from the receptors, once attached, such a mechanism is implicit in the transmission of immunity.

Some of these differences between the two processes may be more apparent than real and careful comparison should result in further insight into both. It is tempting to speculate whether the observation[3] that the absorption of antibody by tissues in passive sensitization *in vivo* or *in vitro* is not commensurate with the capacity to sensitize might not be explained by pinocytosis, as in transmission, that the reversal of established sensitization by non-immune γ-globulin[30] might not depend on a mechanism of release from the receptors and subsequent competition between the free antibody and non-immune γ-globulin for the unoccupied receptors, and that the increase with lapse of time in the difficulty of reversing passive sensitization[22] might be due to the movement of the occupied receptors into the more inaccessible internal regions of the cells. Such rash speculations are warrantable at present only in so far as they may serve to indicate the lines along which further research might prove fruitful.

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VIRUS ISOLATION IN THE ZONAL ULTRACENTRIFUGE

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ZONAL centrifugation has been widely used for virus purification using either rate or equilibrium methods[1]. A limiting factor has been the small capacity of the swinging-bucket rotors generally used. Using a recently developed ultracentrifuge[2,3], I have purified large quantities of virus particles with both methods. This work is described in the present article and also a new technique which combines continuous-flow concentration with equilibrium centrifugation in cæsium chloride, followed by rate-zonal centrifugation in a sucrose gradient. The integrated method is applicable to large-scale virus isolation for research purposes or for vaccine production.

The general principles of the zonal ultracentrifuge have been previously presented[2,3]. The central problems in its development were the elimination of swinging buckets or centrifuge tubes of any sort, provision for sedimentation to occur in sector-shaped compartments, development of rapid methods for establishing and recovering large

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