

developed here has achieved merely the first crack in the olfactory code. The specific anosmia method appears to be a perfectly general procedure, however, applicable to any primary odour. The quantitative definition of a given type of specific anosmia, and its demarcation from all other types of anosmia, would establish a new primary odour. The chemical exploration that I have demonstrated should make it possible to map the exact domain of each new primary. Step by step the whole vocabulary of the olfactory code could be systematically compiled.

Not until it is completely deciphered will the olfactory code develop its maximum utility in scientific and industrial applications, where the majority of odours contain a multiplicity of chemical components belonging to several odour classes.

It would be a considerable help in planning the necessary survey if it were known how many types of specific anosmia may exist. I shall be most grateful to anybody

who volunteers information on any example of "odour blindness" in his experience.

I thank Mrs Delpha Venstrom for assistance, Dr Darrell Althausen for discussion and the entire personnel of this laboratory for participating in the tests. I would like to suggest that, by analogy with Daltonism, we should commemorate Alfred Davis by naming the isobutyric acid anosmia "Davism".

Received December 13, 1966.

<sup>1</sup> Kirk, R. L., and Stenhouse, N. S., *Nature*, **171**, 698 (1953).

<sup>2</sup> Patterson, P. M., and Lauder, B. A., *J. Heredity*, **39**, 295 (1948).

<sup>3</sup> Guillot, M., *C.R. Acad. Sci., Paris*, **226**, 1307 (1948).

<sup>4</sup> Davis, A. R., letter of February 19, 1964.

<sup>5</sup> Amooore, J. E., and Venstrom, D., *J. Food Sci.*, **31**, 118 (1966).

<sup>6</sup> Amooore, J. E., *Cold Spring Harbor Symp. Quant. Biol.*, **30**, 623 (1965).

<sup>7</sup> Zwaardemaker, H., *Arch. Neerl. Physiol.*, **6**, 336 (1922). Ohma, S., *ibid.*, **6**, 567 (1922).

<sup>8</sup> Amooore, J. E., *Proc. Sci. Sect., Toilet Goods Assoc.*, No. 37, Suppl., 1 (1962). Amooore, J. E., *Nature*, **198**, 271 (1963).

## Postnatal Neurogenesis in the Guinea-pig

by

JOSEPH ALTMAN  
GOPAL D. DAS

Psychophysiological Laboratory,  
Massachusetts Institute of Technology,  
Cambridge, Massachusetts

The young of rats and mice are immature at birth. After birth, their brains grow in size and there is also a marked proliferation of cells which become differentiated into neurones with short axons (micro-neurones). The proliferation of similar cells has now been demonstrated in the hippocampus of postnatal guinea-pigs even though these rodents are born with nearly full-size brains.

THYMIDINE-<sup>3</sup>H autoradiography has established that a large proportion of the short axoned neurones (micro-neurones) that form discrete granular layers in brain structures such as the cerebellar cortex and hippocampal dentate gyrus come into existence postnatally in mice<sup>1-3</sup> and rats<sup>4-7</sup>. Mice and rats are characterized by a relatively brief intrauterine period of development, and the young are quite immature, both somatically and behaviourally, for some time after birth. Correspondingly, the brains of these altricial rodents show considerable postnatal growth; in the rat, for instance, the gross weight of the brain increases sixfold (from 0.3 to 1.9 g) from birth to maturity<sup>8</sup>. Is postnatal neurogenesis to be attributed entirely to these characteristics of mice and rats or does it also occur in precocial species? To obtain an answer to this question we have investigated postnatal neurogenesis in a precocial rodent, the guinea-pig. The average gestation period of the guinea-pig is 9 weeks, and guinea-pigs have their eyes open and can stand and run immediately after birth. The brain of the newborn guinea-pig is singularly mature, and there is relatively little weight increase (from 2.5 to 4 g) from birth to maturity<sup>9</sup>.

We injected intraperitoneally a group of newborn (nominally 6 h old) and 6 day old guinea-pigs with tritiated thymidine (dose 10  $\mu$ c./g of body weight; specific activity 6.7 c./mmole; the radiochemical dissolved in isotonic saline, 1 mc./ml.). Single or pairs of animals in both groups were killed 6 h, 12 days and 30 days after injection by cardiac perfusion with 10 per cent neutral formalin. The removed brains were further fixed in formalin, then dehydrated, embedded in 'Paraplast', and serial coronal sections were cut 6 $\mu$  thick. The sections were stained with galloxyanin chromalum and coated for autoradiography in the dark with melted Kodak NTB3 nuclear emulsion. The coated sections were exposed with a desiccant at 5° C for 92 days, and were then developed and processed in the usual manner<sup>10</sup>.

Tritiated thymidine is utilized specifically for DNA synthesis, and therefore those cells that are preparing to

multiply at the time this radiochemical is made available to them become labelled. With autoradiography these cells are easily identified by the blackened silver grains that are situated over the radioactively cell nuclei. The animals with a short survival period after injection (6 h) were used to determine the sites and regional rates of cell multiplication at the time of injection. The animals with intermediate survival after injection (12 days) were used to obtain information about the migration of the labelled cells, and about the rate of their remultiplications (each time a labelled cell multiplies it loses on average half of its labelled DNA). Finally, the animals showing long survival after injection (30 days) were used to establish the destination of the migratory cells and their mode of differentiation. In this paper we shall deal only with results obtained in two brain regions, the dentate gyrus of the hippocampus and the cerebellar cortex.

In the guinea-pigs injected when 6 h old and killed 6 h afterwards, intensely labelled cells were seen in a large concentration, in the vicinity of the hippocampal area, in the relatively thin sub-ependymal layer surrounding the lateral ventricle. Within the hippocampal area many intensely labelled cells were seen in the fimbria and also in the polymorph cell layer (Fig. 1). In addition to the small cells situated in the polymorph cell layer, many of the small cells forming a thin band below the granular layer of the dentate gyrus (the basal germinal zone) were also intensely labelled. Few or no labelled cells, however, were seen in the granular layer itself. For quantitative evaluation of hippocampal neurogenesis we selected in all animals homologous sections of the dentate gyrus in the dorsal hippocampus, and all the labelled cells in this area were counted and classified (Fig. 2). In the animals that were injected at 6 h of age and survived for 12 days afterwards, few intensely labelled cells were seen at any of the sites mentioned, but there was a considerable increase in the total number of labelled cells, with most of them showing much decreased label concentration, indicating a fast rate of continuing cell multiplication during this

period. Increase in the number of labelled cells was pronounced in the polymorph cell layer, and was quite conspicuous in the small cell zone situated below the granular layer, where the majority of the cells present were labelled. A few labelled cells were also seen in the granular layer, but these were generally small, spindle-shaped cells; differentiated granule cells were not labelled. Finally, in the animals that were injected at 6 h of age and survived for 30 days afterwards, there was a considerable reduction in the number of labelled cells in the polymorph cell layer and in the basal germinal zone of the dentate gyrus, combined with a great increase in the number of labelled cells within the granular layer. Many of these labelled cells were large, apparently differentiated granule cells, and were usually situated in the lower half of the granular layer; others were smaller cells which began to assume the shape and appearance of granular neurones. Most of these cells were lightly labelled.

Comparable results were obtained in the guinea-pigs injected when 6 days old which were killed 6 h, 12 days and 30 days afterwards (Fig. 3). In this group of animals many intensely labelled cells were seen after 6 h of survival in the polymorph cell layer; very few in the basal, small-cell zone of the granular layer; none in the granular layer. There was a great increase in the total number of labelled cells after 12 days of survival, and most of these cells showed intermediate or light labelling. The increase was evident in the polymorph cell layer, but was particularly conspicuous in the basal small cell zone below the granular layer. There were a few labelled cells also in the granular layer; these were all small cells. In the animals surviving for 30 days there were many labelled differentiated granule cells in the granular layer; most of these were very lightly labelled.

These results indicate that the primary site of cell multiplication in the hippocampal area is the polymorph cell layer. From here the newly formed cells migrate radially into the basal small cell zone situated below the granular layer, where they continue to multiply at a slower rate. Then from this region they migrate into the granular layer and as they become differentiated into typical granular neurones of this region they cease to multiply. The total number of labelled cells present in the area dentata in both injection groups is smaller in the animals surviving for 30 days than in those surviving for 12 days after injection, as shown in Figs. 2B and 3B; this is presumably because of a continuing fast rate of cell proliferation up to this age (30 and 36 days) which leads to label dilution within many of the cells beyond detectability.

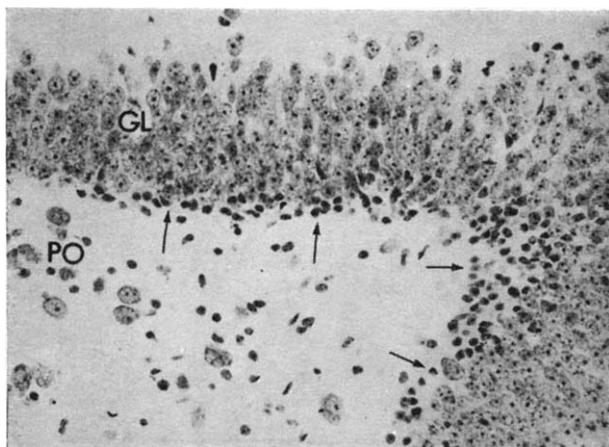


Fig. 1. Photomicrograph of a portion of the hippocampal dentate gyrus in a 30 day old guinea-pig. GL, Granular layer; PO, polymorph cell layer. Arrows point to some of the small, darkly staining cells of the basal germinal zone. These are neuroblasts that migrate into the granular layer and become differentiated there. Stained with galocyanin chromalum. ( $\times c. 170$ .)

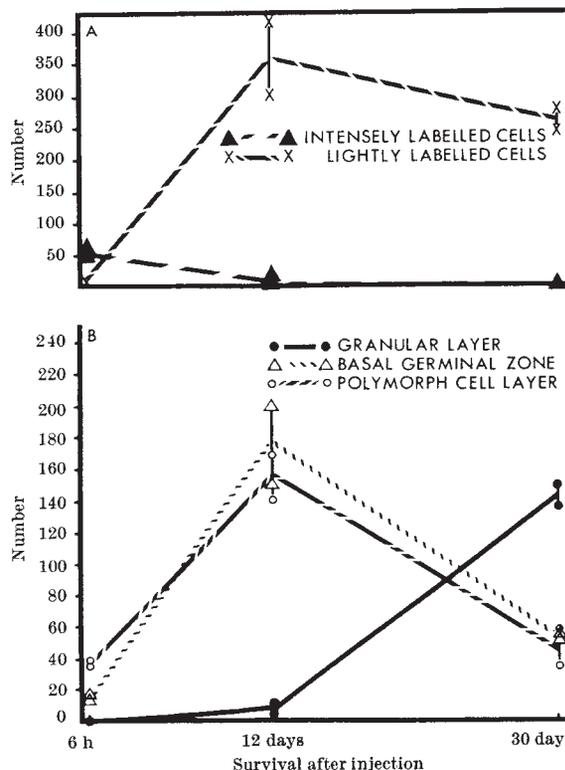


Fig. 2. (A) Classification of all the labelled cells, in terms of concentration of label over cell nuclei, in the sampled region of the hippocampal dentate gyrus as a function of period of survival after injection. The guinea-pigs were injected with tritiated thymidine 6 h after birth. (B) Number of labelled cells in the same animals in different layers or zones of the dentate gyrus.

The foregoing conclusion agrees with our previous results in the rat<sup>4-7</sup> and it indicates that postnatal hippocampal neurogenesis applies not only to precocial but also to altricial rodents. Some differences in the two species should be pointed out. First, the dentate gyrus is considerably more mature in the neonate guinea-pig than in the rat; for in the guinea-pig a large population of differentiated granule cells is present at birth, but few such cells are seen in the newborn rat. In agreement with this, the proportion of labelled (that is, new) cells present in the granular layer of the dentate gyrus in the guinea-pig was much lower than that observed in the rat. In the two groups of guinea-pigs which survived for 30 days after injection an average of 15 per cent (range 11 per cent to 18 per cent) of the total cell population was labelled. In contrast, in rats injected at 6 days of age, with 20 days survival after injection, about 45 per cent of the cells in the granular layer were labelled<sup>11</sup>. Second, postnatal hippocampal neurogenesis is slower and more prolonged in the guinea-pig than in the rat. This is indicated by the following observations. (a) The rate of hippocampal neurogenesis in guinea-pigs in the first week after birth, representing the tenth week of gestation, is much faster than in rats of comparable gestational age (seventh week after birth). (b) In the rat most labelled cells in the granular layer were well differentiated neurones by the twentieth day after injection<sup>4-7,11</sup>; in the guinea-pig many of the cells in this region were still poorly differentiated 30 days after injection. (c) Unlike the situation in the rat, in which the neuroblast zone below the granular layer is in a process of dissolution by the end of the third week after birth, we observed that this zone is prominent in 30-36 day old guinea-pigs (Fig. 1) and is still present in sexually mature young-adult guinea-pigs.

Whereas hippocampal neurogenesis is quite pronounced for some time after birth in the guinea-pig, postnatal cerebellar neurogenesis is minimal in this precocial species.

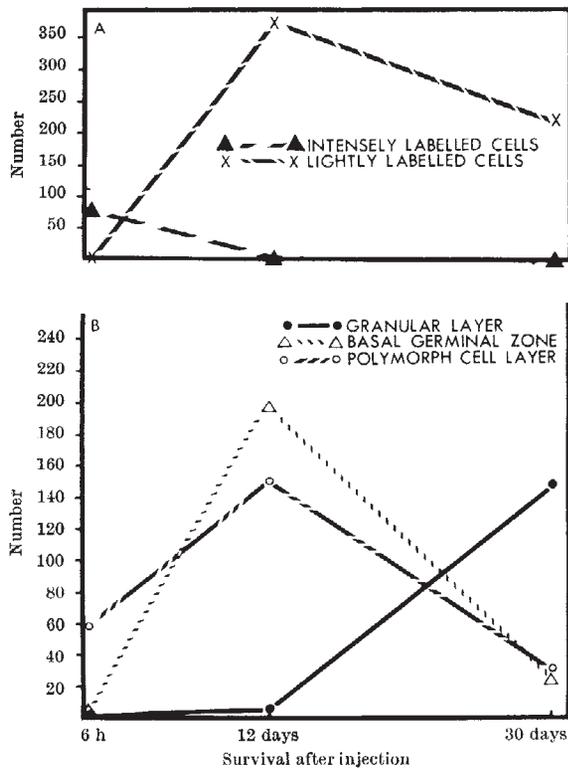


Fig. 3. (A) Classification of labelled cells in the hippocampal dentate gyrus of guinea-pigs that were injected when 6 days old and which survived for different periods afterwards. (B) Number of labelled cells in different layers or zones in the same animals.

In the newborn rat the surface area of the cerebellar cortex is a small fraction of what it becomes in the adult (few of the cerebellar folia are present) and the structure of the cerebellar cortex is embryonic (the molecular and granular layers are practically unformed). About the time of birth, cells begin to multiply very fast in the thick sub-pial germinal zone of the cerebellar cortex, the external granular layer. These cells migrate inwards through the developing molecular layer, past the differentiating Purkinje cells, and into the (internal) granular layer, where they become differentiated into granular neurones. As a result of a large increase of its microneuronal population (consisting of the granule cells of the granular layer, and the stellate and basket cells of the molecular layer) and the concomitant differentiation of the Purkinje cells, the surface area of the cerebellar cortex increases considerably in the rat from birth to the end of the third week. In contrast, the cerebellar cortex of the guinea-pig is well developed at birth. The Purkinje cells are differentiated, and there is a thick molecular and granular layer throughout the cerebellum. A thin (about two to four cells thick) external granular layer is still present in the newborn guinea-pig, but this germinal layer has gone in most regions of the cerebellum in the 6 day old guinea-pig, although remnants of it are still present in some regions.

Wherever the external granular layer was present, however, a large proportion of its cells were labelled with tritiated thymidine, and the process of cerebellar neurogenesis, which is almost at its end in the newborn guinea-pig, is similar to that observed in the rat. For quantitative evaluation of the autoradiograms we selected in one animal from each group a homologous strip of cortex in the uvula, and counted and classified all the labelled cells that were encountered. In the external granular layer over 430 intensely labelled cells were counted in the animal which was injected at 6 h of age and survived for 6 h after injection (Fig. 4B). There were few labelled cells (about thirty) in this animal in the molecular layer, and

even fewer (about fifteen) in the (internal) granular layer. In the animal that was injected at the same time but survived for 12 days after injection, the occasional cells that were still seen in some places in the practically dissolved external granular layer were lightly labelled. In this animal the population of labelled cells increased greatly in the molecular layer (to about 140 cells), and to an even greater extent in the (internal) granular layer (to about 890 cells). In general, the cells of the molecular layer were very lightly labelled; the cells of the (internal) granular layer showed mostly intermediate labelling, many of them were intensely labelled. In the animal which survived for 30 days after injection, there was a decrease in the number of labelled cells in the molecular layer (to about forty cells) and also in the (internal) granular layer (to about 700 cells). In the latter region there was no appreciable change in the number of intensely labelled cells, indicating a deceleration in the rate of remultiplication of the originally labelled cells. Comparable observations were also made in the animals injected at 6 days of age (Fig. 4A) except that there was a general decrease in the number of labelled cells in this group. In the uvula, in which a one to two cell thick external granular layer was still present in the 6 day old animals, 150 intensely labelled cells were counted in the animal surviving for 6 h after injection. Few labelled cells were present in this animal in the molecular or (internal) granular layer. In the animal surviving for 12 days after injection, about 170 lightly labelled cells were counted in the molecular layer and about 680 cells in the (internal) granular layer, with most of the cells in the latter region showing intermediate concentration of label and many intense labelling. In the animal surviving for 30 days after injection there was some reduction in these two layers in the total number of labelled cells, but no change in the number of intensely labelled cells in the (internal) granular layer.

These data indicate similarities and differences in postnatal neurogenesis between the altricial rat and the precocial guinea-pig. The cerebellar cortex in the guinea-pig, unlike that of the rat, is mature at birth and only a small fraction of its granule cell population is formed after birth. The duration of cerebellar neurogenesis in different mammalian species, as judged by the time of dissolution of

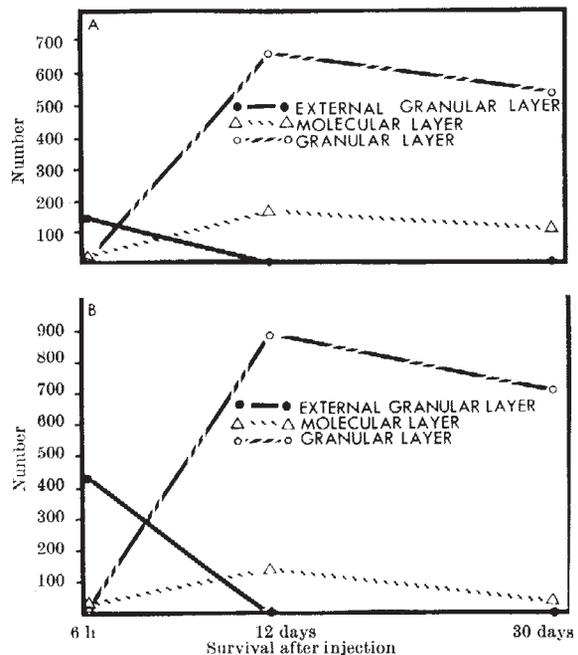


Fig. 4. (A) Number of labelled cells in the three layers of the cerebellar cortex in a strip of the uvula, as a function of survival time after injection, in guinea-pigs injected when 6 days old. (B) The same in guinea-pigs injected at 6 h.

the external granular layer, seems to be correlated with the period that is necessary for the maturation of their locomotor and related skills and also with species differences in the complexity of these skills. Thus, in the rat, the cerebellar external granular layer disappears at the end of the third week after birth; in the cat at the end of the second month; and in man (who is characterized by a very slowly and laboriously acquired bipedal locomotor capacity) by 12–20 months<sup>12</sup>. Accordingly, the cessation of cerebellar neurogenesis soon after birth in the guinea-pig may be related to the circumstance that the limited locomotor capacity of the guinea-pig seems to be developed at birth. In contrast to the cerebellar cortex, postnatal neurogenesis is quite pronounced in the hippocampal dentate gyrus of the guinea-pig. This result suggests that factors other than short gestation period and general immaturity of the brain at birth need to be invoked to account for the prolonged postnatal recruitment of the granule cell population of the hippocampus. Possibly these late-forming elements control some aspect

of behaviour which matures or becomes functional after birth.

This work was supported by the US Atomic Energy Commission. We thank Nicole Poussart for preparing the figures.

Received January 10, 1967.

- <sup>1</sup> Uzman, L. L., *J. Comp. Neurol.*, **114**, 137 (1960).
- <sup>2</sup> Miale, L. L., and Sidman, R. L., *Exp. Neurol.*, **4**, 277 (1961).
- <sup>3</sup> Angevine, J. B., *Exp. Neurol. Suppl.*, **2**, 1 (1965).
- <sup>4</sup> Altman, J., *Anat. Rec.*, **145**, 573 (1963).
- <sup>5</sup> Altman, J., and Das, G. D., *J. Comp. Neurol.*, **124**, 319 (1965). Altman, J., and Das, G. D., *Nature*, **207**, 953 (1965).
- <sup>6</sup> Altman, J., and Das, G. D., *J. Comp. Neurol.*, **126**, 337 (1966).
- <sup>7</sup> Altman, J., *J. Comp. Neurol.*, **128**, 431 (1966).
- <sup>8</sup> Brizzee, K. R., Vogt, J., and Kharetchko, X., *Prog. Brain Res.*, **4**, 136 (1964).
- <sup>9</sup> Himwich, W. A., *Intern. Rev. Neurobiol.*, **4**, 117 (1962).
- <sup>10</sup> Altman, J., in *Response of the Nervous System to Ionizing Radiation* (edit. by Haley, T. J., and Snider, R. S.), 336 (Boston, Little, Brown, 1964).
- <sup>11</sup> Altman, J., *J. Comp. Neurol.*, **128**, 431 (1966).
- <sup>12</sup> Raaf, J., and Kernohan, J. W., *Amer. J. Anat.*, **75**, 151 (1944).

## Effect of pH on $\beta$ -Lactoglobulins

by

H. A. MCKENZIE

W. H. SAWYER\*

Department of Physical Biochemistry,  
Institute of Advanced Studies,  
Australian National University,  
Canberra, A.C.T.

The genetic variants of  $\beta$ -lactoglobulin undergo changes of conformation and molecular size as the pH is varied. These may help to explain some of the changes which occur in milk when it is processed.

ONE of us has reviewed extensively the occurrence, isolation and properties of the  $\beta$ -lactoglobulins of various species<sup>1</sup>. It suffices to recall here that this protein appears to occur only in the milk of ruminants and that it shows genetic polymorphism. Aschaffenburg and Drewry<sup>2</sup> were the first to show the presence of two variants in bovine milk, and Bell<sup>3,4</sup> later demonstrated the presence of a third variant, *C*, in the milk of Jersey cows. Green and Aschaffenburg<sup>5</sup> have shown, by X-ray diffraction studies of crystalline derivatives of the *A* and *B* variants, that each variant is composed of two identical units related by a two-fold axis. An idealized picture emerges of two spheres 17.9 Å in radius, impinging on each other by 2.3 Å at their surface of contact, giving a distance of 33.5 Å from centre to centre. The molecular weight of the dimer unit is 36,000. The amino-acid composition of the three variants is similar, but there are the following residue differences in each monomer unit when referred to the *B* variant<sup>1</sup>: *A* variant: +1 asp, -1 gly, +1 val, -1 ala; *C* variant: +1 his, -1 glu(N).

In 1951 Groves, Hipp and McMeekin<sup>6</sup> found that pooled  $\beta$ -lactoglobulin underwent a large and rapid increase in laevorotation in alkaline solution. Tanford *et al.*<sup>7,8</sup> proposed that this rapid transition is associated with the exposure of two "hidden" carboxyl groups in each  $\beta$ -lactoglobulin dimer. They assumed that no change in the molecular weight of 36,000 is involved and cited unpublished observations of Timasheff and Townsend to support this. As far back as 1936, however, Pedersen<sup>9</sup> had noticed anomalous behaviour in sedimentation velocity and equilibrium experiments near pH 7. Until now, there has been no integrated investigation of changes in the conformation and molecular size of  $\beta$ -lactoglobulins

in the pH range 5–9. We report such work here for three bovine  $\beta$ -lactoglobulins (*A*, *B* and *C*).

Optical rotatory dispersion (ORD) measurements were made in the range 364–578 m $\mu$  for the *A*, *B* and *C* variants prepared by the methods of Armstrong, McKenzie and Sawyer (paper submitted to *Biochim. Biophys. Acta*) and Bell and McKenzie (paper submitted to *Biochim. Biophys. Acta*). The effect of pH value on the specific rotation at 578 m $\mu$ ,  $[\alpha]_{578}$ , and the parameter,  $a_0$ , of the Moffitt–Yang<sup>10</sup> equation is shown in Fig. 1. The parameter,  $b_0$ , of this equation remained sensibly constant for each variant over the pH range 2–9, being  $-67^\circ$  (standard deviation  $6^\circ$ , eighteen measurements),  $-65^\circ$  (standard deviation  $8^\circ$ , twelve measurements),  $-55^\circ$  (standard deviation  $8^\circ$ , eleven measurements) for *A*, *B* and *C*, respectively. Thus it is not surprising that the pH dependence of  $[\alpha]_{578}$  and  $a_0$  is similar for a given variant. Each of the variants shows only slight dependence of  $[\alpha]_{578}$  and  $a_0$  on pH between 2 and 3.5. The *B* and *C* variants show only small dependence of  $[\alpha]_{578}$  and  $a_0$  between pH 3.5 and 4.5. The *A* variant shows strong dependence on pH value between 3.5 and 5, with a minimum in  $-[\alpha]_{578}$  and  $-a_0$  at pH 4.5. This effect is related both to a change in the dimer in which it assumes a specific conformation which is a prerequisite for the formation of octamer, and to the presence of octamer itself (McKenzie, Sawyer and Smith, submitted to *Biochim. Biophys. Acta*). The extent of the dimer–octamer reaction is small for the *A* variant at  $20^\circ$  and is negligible for the *B* and *C* variants<sup>11</sup>. Between pH 4.5 and 7.0, the *B* and *C* variants show a sigmoidal increase in  $-[\alpha]_{578}$  and  $-a_0$ . The same is true for the *A* variant if the effect associated with the octamerization reaction is taken into account. The *A* and *B* variants show a sharp increase in  $-[\alpha]_{578}$  and  $-a_0$  between pH 7 and 9. On the other

\* Present address: The Lister Institute Chelsea Bridge Road, London, S.W.1.