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The Winter Meeting for the Session 1984/85 was held on 17–20 December 1984 at the Department of Anatomy and Biology as Applied to Medicine, The Middlesex Hospital Medical School, London. The meeting was held jointly with the Primate Society of Great Britain and included a two-day symposium on 'Major Topics in Primate Evolution'. The following are the authors' abstracts of the papers and demonstrations submitted to the meeting.

SYMPOSIUM: 'MAJOR TOPICS IN PRIMATE EVOLUTION'

1. Primates: a definition. By R. D. MARTIN (introduced by B. A. WOOD). *Department of Anthropology, University College, London*

Definition of the order Primates, and sub-groups thereof, is intimately linked to phylogenetic reconstruction. It is also beset by similar problems, notably those of identifying derived similarities and of avoiding circular reasoning. Inclusion of tree-shrews in definitions of living primates and inclusion of Plesiadapiformes in definitions of living and fossil primates are best avoided initially, as both are controversial. For various reasons, it is helpful to define living primates (excluding tree-shrews) first and then to consider as separate issues: (1) the relationships of tree-shrews, and (2) the inclusion of particular fossil groups in the order Primates. Previous definitions of living primates have generally been unsatisfactory and are allied to the belief that, in contrast to many other mammalian orders, primates have no general diagnostic features. It will be shown that living primates excluding tree-shrews can be defined quite clearly on the basis of a shared complex of features involving the dentition, cranial morphology, locomotor adaptations, central nervous organisation and reproductive biology. Certain accessory information relating to habitat preference, geographical distribution and body size effects is also relevant.

Following a review of special problems relating to interpretation of the fossil record, the proposed definition of living primates will be refined through reference to the fossil record. In doing so, a major distinction will be drawn between 'fragmentary fossils' whose information content is exhausted by the inference that they are primate-like, and 'substantial fossils' which can contribute additional data for refinement of phylogenetic hypotheses and associated definitions. Fossils currently attributed to the order Primates will be treated in two distinct groups: (1) 'primates of modern aspect', extending back to the early Eocene; (2) 'archaic primates', predominantly from the Palaeocene (viz. the Plesiadapiformes). Evidence linking tree-shrews and 'archaic primates' to fossil 'primates of modern aspect' and their living relatives proves to be quite tenuous. Accordingly, it would seem preferable to define the order Primates to the exclusion of both of these groups.

2. Plesiadapis and primate evolution. By P. D. GINGERICH (introduced by B. A. WOOD). *Museum of Paleontology, The University of Michigan, Ann Arbor, U.S.A.*

Plesiadapis is one of the best known representatives of a large and diverse group of archaic mammals known from the Paleocene of Europe and North America. *Plesiadapis* is interesting from an evolutionary point of view because many fossil specimens have been discovered in successive geological strata, making it possible to trace how plesiadapids changed through time in unusual detail. *Plesiadapis* is interesting from a primatological point of view because it shares some resemblances (primarily dental) with primates, yet it represents a group that is clearly more primitive (in retaining clawed phalanges, etc.) than any primate of modern aspect. *Plesiadapis* itself is too specialized to represent the common ancestor of modern primates in any cladistic sense.

The question of whether *Plesiadapis* and its allies are primates or not, where they rest relative to the 'insectivore-primate' boundary, is probably less important than the question of whether a real boundary can be drawn at all. Much of modern systematic zoology is oriented toward thinking in terms of dichotomies and boundaries – this is a curious perspective for a science embracing evolution, reflecting how little biologists know of the history of life (and, perhaps, the digital basis of modern computing). Classifications impose artificial boundaries for convenience of communication – this does not mean such static boundaries are real. Whether *Plesiadapis* is a primate or not, it is important for primate evolution in representing the grade of evolutionary development of Paleocene mammals (primates included).

3. The relationships of the Tarsiiformes: a review of the case for the Haplorhini. By LESLIE AIELLO (introduced by B. A. WOOD). *Department of Anthropology, University College, London*

The phylogenetic and taxonomic relationships of the Tarsiiformes have been a matter of debate in the primate literature since the middle of 19th century when the first Tarsiiformes from the Eocene began to come to light. This debate gained momentum in the early 20th century when Pocock proposed that the order Primates should be divided into the Haplorhini (anthropoid primates and living tarsiers) on the one hand, and the Strepsirhini (lemurs and lorises) on the other. The case for the Haplorhini rests primarily on the strength of the derived characteristics shared in common by the living tarsiers and the anthropoid primates. This supports the hypothesis that these primates constitute sister groups and, therefore, most probably had a closer evolutionary relationship with each other than with any other living primate. The case against the Haplorhini is drawn primarily from the evidence of the fossil record and is based on two separate arguments. The first is that the Eocene adapids, and not the living tarsiers, are the sister group of the anthropoid primates. The second rejects the sister group status of the adapids and the anthropoids, but argues that the Haplorhini–Strepsirhini dichotomy is of limited utility from a palaeontological point of view. The basic problem stems from the difficulty in assigning Eocene fossil primates either to the Haplorhini or to the Strepsirhini on the basis of robust synapomorphic characteristics.

The case for the Haplorhini therefore rests on two separate questions. Firstly, can the hypothesis that the living tarsiers are the sister group of the anthropoid primates be falsified? Secondly, can the Haplorhini–Strepsirhini dichotomy be usefully applied in the classification of both living and fossil primates? If the hypothesis that the living tarsiers are the sister group of the anthropoid primates can be falsified by either neontological or palaeontological evidence, the case for the Haplorhini would be negated. However, if this hypothesis cannot be falsified, the case for the Haplorhini would rest on the utility of the Haplorhini–Strepsirhini dichotomy in relation to other systems of classification for the primates. These two questions, therefore, form the basis of this assessment of the relationships of the Tarsiiformes.

4. How are platyrrhines related to Old World primates? By A. L. ROSENBERGER (introduced by B. A. WOOD). *Department of Anthropology, University of Illinois at Chicago, Chicago, U.S.A.*

The platyrrhines are of interest in the story of primate evolution because (1) they manifest an extensive arboreal adaptive radiation; (2) they have been confined to South America for nearly all of their 35 million years history; and (3) they have been regarded as a genealogical/structural link between lower and higher primates. In recent years, platyrrhine origins have been revitalised with the geological demonstration that Mesozoic intercontinental connections existed; hitherto, Tertiary land bridges were postulated to explain faunal dispersion across the Atlantic Ocean. From the perspective of systematics, the hypothesis that Neotropical monkeys migrated across a narrow Atlantic can be tested by examining the case for anthropoid monophyly, the relationships between platyrrhines and possible sister-groups in the Old World, and the evidence for endemism.

A rethinking of platyrrhine evolution suggests that the gradational view that the New World monkeys lie between lemur- or tarsier-like primates and catarrhines is incorrect. The morphology of the earliest catarrhines is comparable in form and adaptation to that of some modern platyrrhines. However, even the archaic platyrrhines *Branisella* and *Dolichocebus* are, in ways, more advanced, or derived, than contemporaneous Fayum catarrhines, and thus argues against the notion that known African catarrhines were platyrrhine ancestors. Other Old World fossils

reputed to be primitive anthropoids, such as *Pondaungia* and *Oligopithecus*, exhibit only superficial resemblances to New World monkeys and their significance cannot be properly assessed as yet.

Anthropoids appear to be monophyletic, but our sparse knowledge of palaeodistributions leaves their zoogeography a matter of speculation. That platyrrhines and catarrhines had already evolved some of their dental specialisations by early Oligocene weighs heavily against a transatlantic theory, because each displays similar dietary adaptations yet the catarrhines also exhibit some of their highly canalised dental traits. We are more confident now that platyrrhines are the only known sister-taxon of the catarrhines, but we do not yet know who their immediate ancestor was, or where it lived.

5. The African evidence for early anthropoid evolution. By J. G. FLEAGLE (introduced by B. A. WOOD). *Department of Anatomical Sciences, School of Medicine, State University of New York, Stony Brook, New York U.S.A.*

The eleven primate species from early Oligocene sediments in the Fayum Depression of Egypt provide our only good record of early anthropoid evolution in the Old World. The most striking aspect of this fauna is how different the primates from the Fayum are from later catarrhines. Phyletically they cannot easily be placed in any of the modern anthropoid families. Rather they are very primitive morphologically, and provide a record of early stages in anthropoid and catarrhine evolution that are not represented among extant Old World taxa. In addition, the Fayum anthropoids appear to be adaptively different from later catarrhines in lacking large, folivorous, or terrestrial species. In this regard they are more reminiscent of living and fossil platyrrhines. All available evidence suggests that the Fayum anthropoids were antecedent to the major phyletic and adaptive radiations of modern catarrhines.

6. The evidence from extant catarrhines. By P. ANDREWS (introduced by B. A. WOOD). *Department of Palaeontology, British Museum (Natural History), London*

Predictions are made about the characters present in the ancestral catarrhine morphotype. This is based entirely on the shared characters of the living catarrhine taxa and does not include any input from fossils. There are two reasons for this procedure; firstly, there is a much greater range of characters available to draw upon for the living forms than would be available in the fragmentary fossil record. Because of this, the relationships of living forms can be determined with a greater degree of certainty than would be possible for fossils. Secondly, the framework of relationships demonstrated in this way can be given a phylogenetic perspective by the subsequent addition of fossil evidence, whereas if the fossils were used to determine the relationships as well, this would lead to a circular line of reasoning.

The main purpose in predicting the ancestral catarrhine condition and identifying fossil catarrhines is to try and establish where and when the catarrhines emerged. It will be shown that the earliest known catarrhine primate is *Propliopithecus* from the Oligocene deposits of the Egyptian Fayum, so that the earliest fossil evidence establishes the catarrhines in Africa about 30 million years ago. This is supported by the negative evidence of the lack of any other fossil catarrhines either earlier in time or from anywhere else in the world, because it will be shown that *Pondaungia* and *Amphipithecus* from Eocene deposits in Burma, the only fossils known that could challenge this position, are not demonstrably catarrhine. It is also supported by further circumstantial evidence that later fossil primates from the first half of the Miocene are all clearly related to the living catarrhines and are confined to Africa, the earliest non-African species being middle Miocene pliopithecines and sivapithecines and late Miocene monkeys.

7. Hominoid phylogeny: can a case be made for the Pongidae? By L. MARTIN (introduced by B. A. WOOD). *Department of Anatomy and Embryology, University College, London*

Traditional classification places the great apes (the orang-utan, the gorilla, the chimpanzee and the bonobo) into the family Pongidae, and man and his fossil relatives into the family Hominidae. As a result of molecular studies of living primates, and the influence of the cladistic method of phylogeny reconstruction on the interpretation of morphological evidence, it has

become the consensus that man is more closely related to the African apes than to the orang-utan. However, it has also been proposed that man is more closely related to the chimpanzees than to the gorilla. More recently, it has been suggested on the basis of morphological similarities, and especially the shared possession of molar teeth with thickened enamel, that man is more closely related to the orang-utan than either is to the African apes. This paper reassesses the molecular and the morphological evidence for relationships within the great ape and human clade and attempts to reconstruct the morphology of the common ancestors of the groups within that clade.

The weight of molecular and morphological evidence supports the interpretation of the great ape and human clade as a monophyletic group. Within that group the bonobo is the sister species of the chimpanzee, and both are equally closely related to the gorilla. Man is more closely related to all of the African apes than to the orang-utan. Two families are recognised for the great ape and human clade: the Hominidae (comprising the Gorillinae for the African apes and the Homininae for *Homo sapiens* and its fossil relatives) and the Pongidae for the orang-utan and *Sivapithecus*.

8. Molecular sequences and anthropoid phylogeny. By A. E. FRIDAY and M. J. BISHOP. *Department of Zoology, University of Cambridge*

There have been many attempts to use the data of molecular sequences to estimate the evolutionary relationships of anthropoid primates. Most work has employed so-called 'parsimony' methods to examine protein and nucleic acid sequences. It is clear that the model of evolutionary change implied by the parsimony approach involves unrealistic assumptions about the nature of molecular evolution. Models based on probabilistic processes of change can be defended as more realistic, and estimations can be carried out under these models using the method of maximum likelihood. It is emphasised that different models of the process of evolutionary change may lead to different best estimates of phylogenetic trees for the same data. Commitment to a model of change is therefore the first step to be taken.

It is a feature of the approach advocated that the different amounts of support for different phylogenetic trees, based on the same data, can be compared. Sometimes the estimates reveal that there is not enough information to resolve problems of the order of branching in phylogenetic trees. A particularly resistant case has been the phylogenetic tree for living hominoids, and we reassess reconstructions made from molecular data.

9. *Homo* and *Paranthropus*. By M. C. DEAN (introduced by B. A. WOOD). *Department of Anatomy and Embryology, University College, London*

The affinity of *Paranthropus* and the 'gracile' australopithecines has been stressed by many workers and some have suggested that many of the obvious morphological differences between them can be explained simply as variation resulting from a difference in body size. Nevertheless there a number of anatomical features that *Paranthropus* shares with *Homo* which are not found in the 'gracile' australopithecines. Among these characters are the retruded face, small anterior tooth size, an identical eruption pattern of the permanent dentition, a flexed cranial base in the sagittal plane and inwardly rotated petrous temporal bones, and a shared pattern of muscle markings in the region of the upper pharynx.

Following the attribution to *Homo* of fossil hominid specimens from Koobi Fora with cranial crests and broad retruded faces, and the reattribution of specimens from Swartkrans previously assigned to *Paranthropus* to *Homo*, it has become even more important to reassess the characters that these two taxa share in common. They could be explained simply as examples of parallel evolution occurring for different functional reasons in *Homo* and *Paranthropus*. It remains, however, possible that *Paranthropus* and *Homo* share derived characters inherited from an (as yet unidentified) common ancestor of both. The assessment of the relative merits of these two proposals requires either that a satisfactory explanation for such a large number of parallelisms occurring in *Homo* and *Paranthropus* be found or that a suitable ancestral form be identified. Whatever the reasons, it is clear that as well as showing many features of the australopithecines, *Paranthropus* also shares many cranial and dental traits with *Homo*.

10. Dental trends in the australopithecines. By F. E. GRINE (introduced by B. A. WOOD). *Department of Anthropology, State University of New York, Stony Brook*

A variety of allometric and ecological arguments have been employed in nearly forty years of debate over the evolutionary and taxonomic distinctiveness of the so-called 'gracile' and 'robust' australopithecines. While some have maintained that these 'gracile' and 'robust' forms should be accorded separate generic status (i.e. *Australopithecus* and *Paranthropus*), others have suggested that the differences between them are merely correlates of allometric scaling.

Compared to cheek tooth sizes, the canines and incisors of the 'robust' australopithecines are relatively smaller than 'gracile' homologues. The relative reduction of anterior tooth size appears to be related to functional, rather than to simple allometric differences between the 'robust' and 'gracile' hominids. Comparisons amongst the mesiodistal dimensions and the buccolingual diameters of dm_2 and M_1 talonids and trigonids reveal the distal moieties of these molars to be relatively broader in 'robust' than in 'gracile' specimens. These differences seem also to be related to functional, as opposed to allometric factors. The dentitions of 'gracile' and 'robust' australopithecines differ, too, in a variety of discrete, non-metrical features, and a number of the uniquely derived characters evinced by the 'robust' specimens can be related to differences in masticatory function.

It is argued that the dental evidence warrants the 'gracile' and 'robust' australopithecines being accorded separate generic status.

11. Australopithecines: grade or clade? By B. A. WOOD and A. T. CHAMBERLAIN. *Department of Anatomy and Biology as Applied to Medicine, The Middlesex Hospital Medical School, London*

Most definitions of, and references to, australopithecines have assumed that the name refers to a grade of small-brained hominids with relatively large cheek teeth, and four taxa. *A. afarensis*, *A. africanus*, *A. robustus* and *A. boisei* are currently included in that definition. The few cladistic analyses of early hominids which have been carried out have mostly made it plain that *A. boisei*, and to a lesser extent *A. robustus*, show a series of apparently unique features which set them apart from other hominids. Opinions are divided about *A. africanus*. Some workers consider that it shares enough features with the 'robust' australopithecines to be regarded as their sister group, while others take the view that the majority of its character states suggest that *A. africanus* is the sister group of *Homo*. However, if unique features can be cited which link *A. africanus* and the two 'robust' taxa in a clade, is the fourth taxon, *A. afarensis*, more closely linked to this clade, to the *Homo* clade, or does it fail to show any particular link with either?

The apparent synapomorphies of the 'robust' australopithecines are reassessed and the extent to which *A. africanus* shares character states with *A. boisei* and *A. robustus* are reviewed. The review examines the proposal that the apparent affinities between *A. africanus* and the 'robust' taxa reflect a series of intercorrelated features all related to a similar, and perhaps convergent, dietary adaptation. Finally, cladistic relationships are used to both reassess the affinities of *A. afarensis* and to construct a broadly-based cladogram of early hominid groups.

12. The credibility of *Homo habilis*. By C. B. STRINGER (introduced by B. A. WOOD). *Department of Palaeontology, British Museum (Natural History), London*

The species *Homo habilis* was created by L. S. B. Leakey, Tobias and Napier on the basis of cranial and post-cranial material (aged approx. 1.9–1.6 myr) from Beds I and II, at Olduvai Gorge, Tanzania.

Recent field work in Africa has brought further claims for the presence of the species *H. habilis* from South Africa (Sterkfontein and Swartkrans), Kenya (e.g. Koobi Fora), Ethiopia (Omo) and Olduvai Gorge itself. Problems with the definition of the species began with the morphological range of the Olduvai material itself, and workers such as Brace and Wolpoff have never accepted the validity of the species, preferring instead to allocate individual specimens to *Australopithecus* or *Homo erectus*. Difficulties have been compounded by the tendency for other specimens to be allocated to the taxon on the assumption that it represents a grade of hominid intermediate between Pliocene *Australopithecus* and Pleistocene *Homo*, rather than by specific

comparisons with the original Olduvai *H. habilis* material. One of the few attempts at such comparison led Groves and Mazák (Cas. Miner. Geol., 20, 1975) to propose an additional hominid species, *H. ergaster*, for some of the Koobi Fora material, but unfortunately the necessary delineation of this species from *H. erectus* was not carried out at the same time.

Of the material often considered to belong to the species *H. habilis*, there are crania with a wide range of facial form and endocranial volume (known range at least 515–752 ml), mandibles with size and robusticity overlapping the ranges of *A. africanus* and even *A. robustus*, and dentitions which differ considerably in character from that originally proposed for the species. Workers such as Walker and Wood have argued that there are probably at least two hominid species present within this range of material, but there is no agreement as to which specimens should be retained within the genus *Homo*, and whether there is a need for the creation of one or more additional hominid species. Cladistic methods of study are being applied to some of these fossils for the first time, and the results so far are reviewed. Using other workers' data on the original fossils and my own data gathered from available casts, I intend to examine the cranial material allocated to *H. habilis* for the presence of particular synapomorphies identified for the genus *Homo* (*H. erectus* and *H. sapiens sensu lato*) with the aim of clarifying how many groups may be represented.

13. The origin and fate of *Homo erectus*. By A. BILSBOROUGH and B. A. WOOD*. *Department of Physical Anthropology, University of Cambridge and *Department of Anatomy and Biology as Applied to Medicine, The Middlesex Hospital Medical School, London*

The number of hominid specimens included within *Homo erectus* has increased very considerably over the last two decades. Such specimens are geographically and chronologically diverse and show considerable morphological variation, so that the taxon now shows marked intra-specific variability. While several evolutionary interpretations have been offered to account for this diversity, some workers have questioned the integrity of the taxon and expressed the view that 'erectus' has become a heterogeneous 'dustbin' category to which a variety of Lower and Middle Pleistocene hominid specimens have been uncritically assigned. Other attempts have been made to define more closely both the characteristics and phyletic position of *H. erectus*, and the 'consensus' view of it as a chronospecies of *Homo*, with *H. erectus* populations directly ancestral to *H. sapiens*, has recently been challenged by several workers.

This paper summarises the morphological features of Pleistocene hominids definitely, or provisionally, assigned to *Homo erectus*, with particular emphasis on chronologically based and terminal specimens. It reviews alternative characterisations of the taxon based upon different systematic and phyletic methodologies, and considers their utility for hominid evolutionary studies.

14. The origin of *Homo sapiens*: the genetic evidence. By J. S. JONES (introduced by B. A. WOOD). *Department of Genetics and Biometry, University College, London*

Developments in the techniques of analysing proteins and DNA make it possible for the first time to obtain objective estimates of the extent to which Man shares genes with his close relatives, and of the degree to which geographically separated human populations differ from each other. In principle it is also possible to estimate the evolutionary trees which link the evolving lineages and (by assuming that molecular evolution proceeds at a constant rate) to measure the dates of divergence of these lineages from each other. There are, however, methodological and statistical ambiguities which have led to disagreements between the evolutionary patterns deduced from molecular genetics, and those which come from more traditional studies of primate evolution. An attempt is made to discuss what molecular biology can and cannot tell us about human evolution.

15. The origin of *Homo sapiens*: the fossil evidence. By D. R. PILBEAM (introduced by B. A. WOOD). *Department of Anthropology, Peabody Museum, Harvard University, Cambridge, Massachusetts, U.S.A.*

The evolutionary transition to modern humans is one of the most dramatic of all steps in hominid evolution, and the only one that is reasonably well documented both anatomically and

archaeologically. The transition took less than 100000 years, perhaps substantially less, and both 'before' and 'after' stages are well sampled. At least for the Neanderthal type of 'archaic sapiens', skeletal part representation is excellent so that we have reasonable knowledge of intra- and inter-sexual variation. Other archaic groups are becoming better known.

The transition to modern humans was essentially complete in many (?most) parts of the Old World by just over 30000 years ago, with local completion apparently being heterochronous. The start of the transition is less clear: it began no more than 100000 years earlier, perhaps much later. The pattern of the transition is as equivocal as its timing and it is not possible to decide among several alternatives. Nor does the very abundant archaeological record (yet) help. It is likely that at least an order of magnitude more information will be necessary to settle this micro-evolutionary issue.

Attention is increasingly being paid rather to the more tractable question of the behavioural meaning of the anatomical and archaeological changes from archaic to modern sapiens. Modern patterns evolved relatively rapidly following a much longer period of slow change and stasis. Possibly, changes in information storage and transmission played important roles in this transition. The morphological-archaeological changes mapping behavioural change may not necessarily map genetical change exactly.

Since this last great hominid transition is the best known of all, it deserves close study as a transition. In addition these archaic ancestors of modern humans represent a long-lasting adaptive phase immediately preceding us that both can and must be better understood.

A case can be made that the nomen *H. sapiens* should apply only to hominids for which modern behavioural patterns can reasonably be inferred; another name would then be needed for 'archaic *H. sapiens*'.

COMMUNICATIONS

16. Identification, ultrastructure, and synaptic relationships of the axon terminals of neurons in the superior colliculus that project to the ventral lateral geniculate nucleus: HRP-EM studies in the adult rat. By ALISON M. TAYLOR and A. R. LIEBERMAN. *Department of Anatomy, University College London and Institute of Anatomy, University of Aarhus, Denmark* (Fig. 1)

The ventral lateral geniculate nucleus (LGv) receives retinal input and projects to a variety of subcollicular structures, among them the superior colliculus (SC) (e.g. Brauer & Schober, *Exp. Brain Res.* **45**, 1982) from which the LGv also receives a reciprocal projection (Reese, *Brain Res.* **305**, 1984). We are studying these connections by anterograde and retrograde tracing with HRP, after stereotaxically guided injections of the tracer into the SC of anaesthetised animals. In this communication we focus on the terminals of the axons projecting to LGv from SC, and describe, for the first time in any species, their ultrastructural characteristics and synaptic organisation.

By LM (TMB method) the projection from SC terminates in the most superficial part of the lateral (external) retino-receptive division of the LGv, where the terminals overlap with the cell bodies of large, retrogradely labelled neurons whose axons project to SC.

By EM (Hanker-Yates method) small to medium sized labelled terminals, containing variable amounts of HRP and with small, dark, mitochondria, are readily identified in the superficial part of LGv (LT, Fig. 1A and B). The labelled terminals contain predominantly spherical, lucent synaptic vesicles and some also contain larger dense-cored vesicles (arrows in Fig. 1A and B). All establish Gray type I (asymmetric) synaptic contacts (arrowheads, Fig. 1A and B), outside regions of complex (glomerular) neuropil, chiefly with non-vesicle-containing dendritic shafts of variable diameter. Some also establish synaptic contact with spine-like appendages derived from such dendrites, or with cell bodies, including labelled cell bodies of neurons projecting to SC.

Thus the input from the SC to the LGv is directed predominantly to the dendrites of (presumptive) projection neurons. The fine structural characteristics of their terminals and synaptic contacts suggest that they are excitatory, and that the SC-LGv input is facilitatory with respect to the output of LGv projection cells.

We thank the British and Danish Medical Research Councils for financial assistance.

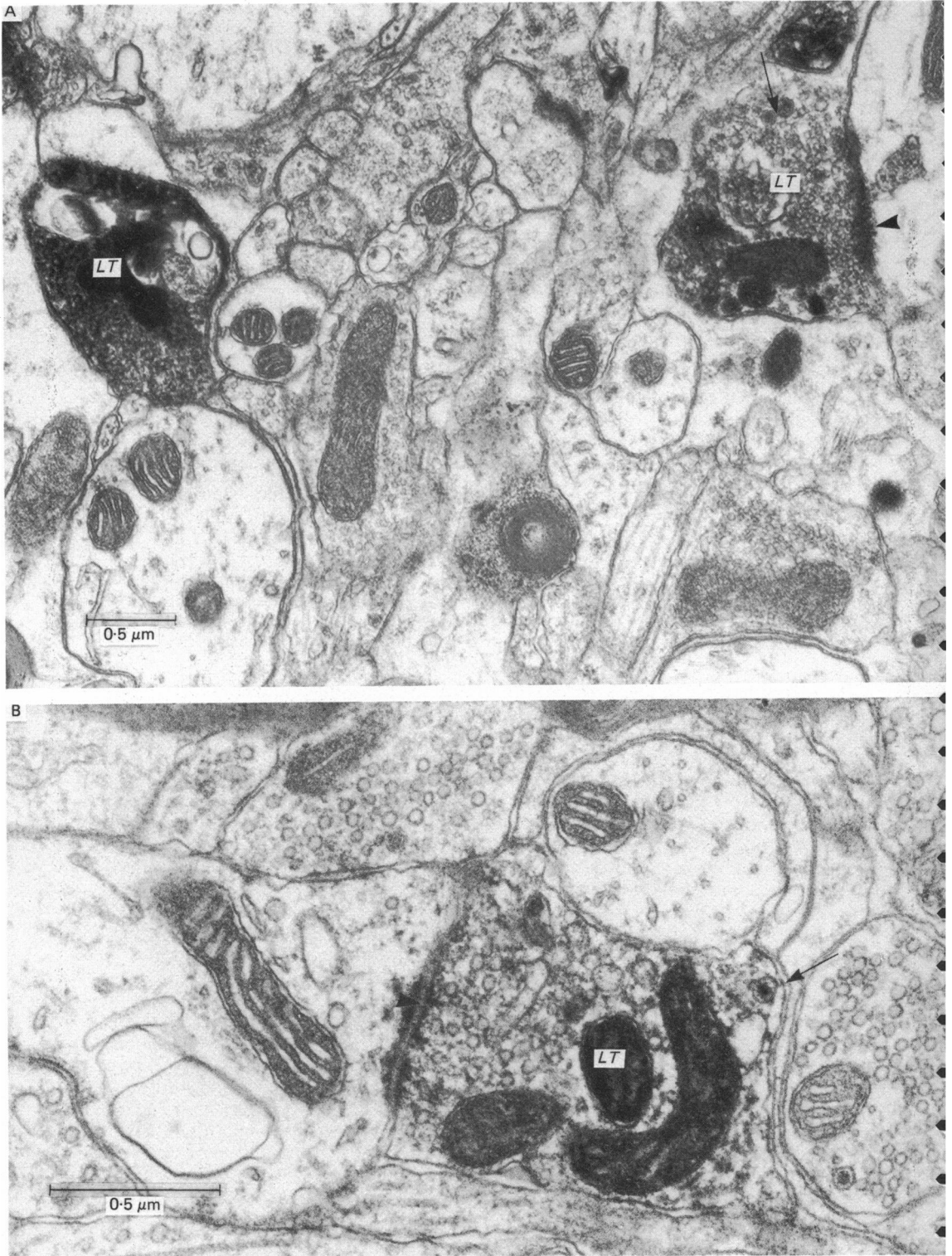


Fig. 1

- 17. Loss of neurons from the locus caeruleus of aged mice.** By K. A. RAO (introduced by R. R. STURROCK). *Department of Anatomy, University of Dundee, Dundee, Scotland*

There have been a number of reports describing loss of neurons from the locus caeruleus (LC) of the ageing human brain. Neuronal loss occurs from the LC of mentally normal patients over the age of 65 and a much more substantial loss of LC neurons occurs in degenerative disorders such as Alzheimer's disease and Parkinson's disease. The only quantitative histological study carried out in rodents has shown that there is no loss of neurons from the LC of the ageing Fisher 344 rat (Goldman & Coleman, *Neurobiol. Ageing* 2, 1981) although there is a slowing down of LC activity in old rats (Olpe & Steinmann, *Brain Res.* 251, 1982).

The ASH/TO strain of mice has a modal lifespan of 22 months. Sets of serial sections were available from brains of ASH/TO mice aged 6, 9, 12, 15, 22, 25, 28 and 31 months. These consisted of right half brains sectioned at 6 μm in the sagittal plane stained with Lapham's stain and left half brains sectioned at 6 μm in the coronal plane stained with H & E. Three sets were available at each age.

Counts of LC neurons were carried out in the sets of sagittal sections and showed a statistically significant loss of neurons in the oldest animals. There was a wide degree of individual variation between 16 and 15 months but up to 25 months of age the mean number of LC neurons at each age never fell below 1325 whereas at 28 and 31 months the mean number was 1009 ± 60 and 854 ± 176 respectively.

These results were normalised and compared with the available human data using a mathematical method similar to that employed by Wree *et al.* (*Anat. Embryol.* 160, 1980). The pattern of LC neuron loss from the ASH/TO mouse brain corresponds very closely with that found in the normal ageing human brain and suggests that this strain of mouse may be a useful model for further studies of the ageing central noradrenergic system.

- 18. Alterations in cell number in the brains of aged mice: an example of plasticity or different populations?** By R. R. STURROCK. *Department of Anatomy, University of Dundee, Dundee, Scotland*

The ASH/TO strain of mice has a modal lifespan of 22 months (Sturrock, *Neuropath. Appl. Neurobiol.* 5, 1979) but by setting up a large colony survivors were found up to 31 months of age. The quantitative histological studies referred to above were continued at 25, 28 and 31 months of age. The rostrocaudal length of the corpus callosum and rostral part of the anterior limb of the anterior commissure was greater from 22 to 31 months than from 6 to 18 months and the transverse length of the anterior commissure was greater from 25 to 31 months than from 6 to 22 months. The number of glia in all parts of the anterior commissure fell between 9 and 18 months then increased substantially between 22 and 25 months to reach, or exceed the 9 months level. A significant increase in glial number was also found in the neostriatum, indusium griseum and subependymal layer between 18 and 22 months.

There was no evidence of neuronal loss from the indusium griseum or neostriatum at any age although a significant loss of neurons from the locus caeruleus has been found at 28 and 31 months in the same mice (Rao, *J. Anat.*, 1985). Glial number declines in the subependymal layer between 22 and 31 months and in the neostriatum between 28 and 31 months but remains constant in the anterior commissure and indusium griseum.

These changes either represent a substantial degree of plasticity in glial number during ageing or raise the possibility that the group of mice which survive beyond the modal lifespan are a different population. Evidence in favour of the latter interpretation includes the apparently larger brains and the substantially greater number of glia in both white and grey matter found in mice between 18 and 25 months of age.

- 19. Fibre connections of the periaqueductal grey in the rat studied by horseradish peroxidase labelling.** By S. MENON (introduced by V. NAVARATNAM). *Department of Anatomy, University of Cambridge*

The periaqueductal grey (PAG) is an extensive region and preliminary light microscope studies, supplemented by transmission electron microscopy, confirm that it may be divided into a magno-

cellular lateral nucleus and a smaller parvicellular part; the parvicellular component may be further subdivided into nucleus dorsalis and nucleus medialis.

Although it is widely believed that the PAG modulates the transmission of pain, its precise fibre connections have not been definitively determined. In this study, its efferent projections to the thalamus and nearby diencephalic nuclei were studied by the stereotaxically guided introduction of Sigma VI horseradish peroxidase (HRP) in 20 young adult fully anaesthetised rats. After 48 hours these were perfused with Karnovsky fixative and the brains were serially sectioned on the freezing microtome and stained, using diaminobenzidine tetrahydrochloride as chromogen. In a further six animals, HRP was introduced into the PAG itself so as to study the afferent connections.

Injections into the anterodorsal and paratenial nuclei of the thalamus result in no labelling of PAG neurons; injections into the lateral septal nucleus also failed to demonstrate connections. However, injections into the lateral thalamic nucleus, parafascicular nucleus, paraventricular nucleus and dorsomedial nucleus showed that these receive projections from the magnocellular lateral nucleus of the PAG while injections into the habenular nuclei or into the pretectal region demonstrated connections from the nucleus dorsalis. A substantial injection site covering the ventral and dorsomedial thalamic nuclei produced labelling in both the lateral and medial nuclei of the PAG. Control experiments to discount effects of uptake along the micropipette track were carried out in which HRP was instilled into the cortex overlying the thalamus but these caused no labelling in the PAG.

Afferent connections to the PAG are received from numerous midbrain structures such as the reticular formation and from diencephalic structures especially the hypothalamus.

20. A double retrograde fluorescent tracer study of axonal branching within the olivocerebellar projection. By S. M. WHARTON* (introduced by J. N. PAYNE). *Department of Anatomy and Cell Biology, University of Sheffield and *Human Morphology, University of Southampton*

Axonal branching within the olivocerebellar projection has been investigated using an anatomical technique. In one group of rats ($n = 9$) diamidino yellow (DY) was injected rostrally, and true blue (TB) caudally in the mid-sagittal plane of the cerebellar vermis of each animal. A second group ($n = 7$) had a rostral injection of DY and a caudal injection of TB, in the same parasagittal plane, laterally in the right cerebellar hemisphere of each animal. A third group ($n = 4$) was injected with DY rostrally in the vermis and, in the same coronal plane, with TB in right hemisphere of each animal. All injections were 50 nl of a 2% suspension and were carried out under general anaesthesia. After 4 days the cerebellum and the brainstem were sectioned in a cryostat and viewed with a fluorescence microscope.

The animals injected in the mid-sagittal plane had DY labelled neurons laterally and TB labelled neurons medially in the caudal part of the medial accessory olive. Where the two labelled cell groups overlapped, double-labelled neurons were present. Animals injected in the same parasagittal plane of the cerebellar hemisphere had DY labelled neurons laterally and TB labelled neurons medially in the principal olive and double-labelled neurons in an intermediate position. The animals injected in the vermis and hemisphere had widely separated labelled cell groups in the inferior olive with no double-labelled neurons.

These results indicate that axons of some inferior olivary neurons branch within parasagittal bands and that their cell bodies lie at the junction of populations of neurons projecting to different regions within the same band. By contrast no evidence was found for olivary cells branching to innervate different regions within the same coronal band.

21. Immunofluorescence studies on the brain of the honeybee, *Apis mellifera*. By M. J. NOBLE (introduced by A. D. HOYES). *Department of Zoology, Queen Mary College and Department of Anatomy, St Mary's Hospital Medical School, London*

The nervous system of the honeybee has been used extensively as a model system to investigate correlations between neuronal properties and animal behaviour. It is only recently, however, that biogenic monoamines have been identified and localised in the honeybee brain (Mercer *et al. Cell Tiss. Res.* 234, 1983). The object of this study was to extend current knowledge of the neurotransmitters present in the honeybee brain using immunofluorescence procedures on frozen sections of adult brains.

Standard immunofluorescence techniques were used on formaldehyde-fixed brains to test antisera raised against 5-HT, GABA and a wide range of small peptides and peptide hormones. The distribution of immunoreactivity was compared with reduced silver and Golgi preparations to confirm the anatomical localisation of antigenic sites.

There were considerable amounts of immunoreactivity in bee brains tested with antisera against 5-HT, GABA and CCK. Plexuses of fibres immunoreactive to 5-HT extended throughout the neuropil of the protocerebrum, deutocerebrum, optic lobe and sub-oesophageal ganglion. A small number of immunoreactive cell bodies were associated with these fibres in the midbrain and suboesophageal ganglion.

GABA-like immunoreactivity was found mainly in the visual system. There were large amounts of immunoreactivity in many cell bodies around the second optic ganglion and in a few cell bodies associated with the first and third optic ganglia and the ocelli. The first optic ganglion and the central body were the only regions where immunoreactivity was present in the neuropil.

The neuropil of the mushroom bodies and central body contained large amounts of CCK-like immunoreactivity. In the mushroom bodies, it was confined to the peduncular and lobular regions. The distribution of CCK-like immunoreactivity closely resembled that of several biogenic monoamines, and further studies are being undertaken to determine whether the peptide is localised within aminergic neurons.

22. Increased vasoactive intestinal polypeptide in central terminal areas of axotomised nerves is of primary afferent origin. By S.A.S. SHEHAB and M. E. ATKINSON. *Department of Anatomy and Cell Biology, University of Sheffield.*

Following peripheral sciatic nerve axotomy, vasoactive intestinal polypeptide (VIP) increases dramatically in the central terminal areas of the nerve from which other peptides are depleted by the operation (Shehab & Atkinson, *J. Anat.* **13**, 1984). VIP may originate from collateral sprouting of adjacent undamaged nerves, intrinsic sources in the spinal cord or from injured nerves themselves. These possibilities were investigated by section of other nerves terminating in the same segments of the spinal cord and dorsal rhizotomy of appropriate nerve roots.

Under ether anaesthesia, the sciatic, saphenous, cutaneous or pudendal nerves were cut distal to the sensory ganglion. In a second group of rats, laminectomy was performed under pentobarbitone sodium anaesthesia and the dorsal roots of the third, fourth, fifth or sixth lumbar nerves were cut. Fifteen or thirty days later the animals were perfusion-fixed and the lumbar segments of the spinal cord were stained for VIP, substance P (SP), somatostatin (SOM) and cholecystinin (CCK) using unlabelled antibody immunohistochemistry.

Following peripheral nerve section, SP, SOM and CCK staining in the dorsal horn of the third to fifth segments of the lumbar spinal cord was abolished from the corresponding terminal areas but VIP increased to replace the depleted peptides in these areas. After dorsal rhizotomy, staining for all neuropeptides, including VIP was abolished from the dorsal horn.

These results demonstrate that VIP only increases if the dorsal root is intact indicating a peripheral origin of VIP. Section of adjacent peripheral nerve branches produced a greater increase in VIP than seen after sciatic nerve section suggesting VIP does not originate from collateral sprouting. In normal animals, VIP is not readily detectable in dorsal root ganglia but after peripheral axotomy, dense VIP immunoreactivity is present in these cells indicating that VIP metabolism in the axotomised cell bodies increases in response to peripheral axotomy.

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23. Antibodies to neuropeptides applied *in vitro* are localised in cell bodies in areas of high neuropeptide receptor density. By SUSAN E. DOUGHTY (introduced by M. E. ATKINSON). *Department of Anatomy and Cell Biology, University of Sheffield*

Antibodies have been used to neutralise endogenous neuropeptides to assess their physiological and behavioural effects but their site of action is unknown. Anti-substance P antibody was localised after injection into brain slices maintained *in vitro* by application of secondary antibody to fixed sections of brain slices.

Cervical spinal cord (500 μm slices) and brainstem were prepared from brains rapidly removed

from decapitated rats and maintained in oxygenated artificial CSF. Of anti-substance P antibody 50 nl was injected into areas of either high SP terminal density, high SP receptor density or absence of SP. After an hour in ACSF the slices were fixed and 25 μ m cryostat sections were incubated in fluorescein-conjugated anti-rat antibody. Control slices were injected with normal rat serum, secondary antibody or primary antibody adsorbed with SP. Non-injected slices maintained in identical conditions were stained by standard indirect immunofluorescence for comparison.

The normal distribution of SP-positive nerve terminals was observed in the cervical spinal cord and brainstem but no fluorescent cell bodies were observed in non-injected slices. In slices injected with anti-substance P antibody, SP-positive cell bodies were observed in the raphe and interpeduncular nuclei; no other structures demonstrated SP-immunoreactivity. Control slices injected in identical sites were non-reactive.

Injection of anti-SP antibody into viable brain slices showed immunoreactive cell bodies in specific nuclei of the brainstem. Antibodies are normally excluded from intact viable cells and the possibilities of passive ingress of antibody into dead cells in the preparation or non-specific endocytosis can be excluded by specificity of staining. Specific uptake of antibody is suggested which may occur through receptor-mediated endocytosis since the areas in which cells stain possess a high density of SP receptors.

24. A study of the potential neurotoxic effects of capsaicin on primary afferent neurons in the bird.

By J. B. BALL (introduced by C. R. VAILLANT). *Department of Veterinary Anatomy, University of Liverpool*

In rodents the selective neurotoxicity of capsaicin for small diameter primary afferent neurons, that is mainly unmyelinated (including peptidergic) afferents, has made it a valuable neurobiological tool. In particular, the almost total loss of unmyelinated afferents following neonatal capsaicin treatment has enabled peripheral peptidergic afferents to be distinguished from peripheral efferents, containing the same peptide.

In birds, however, the effects of capsaicin on primary afferent neurons has not been examined. In the present work, the right dorsal root of the third spinal component of the brachial plexus was studied in six 2-3 months old birds (*Gallus gallus domesticus*), three of which had received a subcutaneous injection of capsaicin (100 mg/kg) within two days of hatching. In addition, the peptidergic innervation of the upper gastrointestinal tract, a region where there is electrophysiological evidence for a sensory innervation, was examined in these birds immunohistochemically.

Dorsal roots from capsaicin-treated birds showed no light or electron microscopic evidence of axonal degeneration. A multiple stage sampling method for estimating numbers of axons in these dorsal roots was developed, and results indicated no significant difference between the populations of myelinated axons 4 μ m and larger in diameter, myelinated axons less than 4 μ m in diameter, and unmyelinated axons in capsaicin-treated and control birds. Immunohistochemical studies revealed no apparent differences in the substance P-, met-enkephalin-, somatostatin- and VIP-immunoreactive innervation of the crop, proventriculus and gizzard in these two groups of birds.

The results indicate that capsaicin does not cause degeneration of primary afferent neurons in the bird. Whether this is due to protection provided by the relative maturity of the nervous system of newly hatched chicks compared with that of neonatal rodents, or to a general insensitivity in birds to capsaicin, is not known. Evidently this experimental tool cannot be used to distinguish between peptidergic afferent and efferent fibres in peripheral avian tissues.

25. Prenatal development of the retina in rat and man. By A. D. FOX (introduced by A. J. PALFREY). *Department of Anatomy, Charing Cross and Westminster Medical School, London*

The early stages in the development of the mammalian retina may be recognised by differentiation of the nuclei between the three primitive layers, the ependymal, mantle and marginal zones. In the human retina the mantle zone is subdivided into inner and outer nuclear layers by the transient fibre layer described by Chievitz in 1887, but this layer has not been identified in the rat.

In this study differentiation of the retina has been followed from the 34th day to the 11th week of gestation in the human and from the 13th to the last (21st) day of gestation in the laboratory rat. A variety of fixatives was used and serial sections examined.

Evidence of nuclear differentiation was detected on day 15 in the rat and at the comparable 20–25 mm stage in the human. Early proliferation of the peripheral ependymal zone was conspicuous in both species. Thereafter, in the rat retina, a gradient of maturation from the central to the peripheral zones was seen, whereas in the human maturation spread both centrally and peripherally from the inner nuclear layer of the mantle zone.

A transitional zone appears within the rat mantle zone between the 16th and 19th days. In the human the transient fibre layer is present from the 20 mm to the 30 mm stage. This transitional zone in the rat is identified with the transient fibre layer in man.

Axons of the primitive ganglion cells were identifiable on the 15th day, thus preceding the migration of ganglion cell precursors into the anuclear maginal zone. This migration was seen on day 17 in the rat and at the 16 mm stage in man. Subsequent enlargement of the nerve fibre layer was inversely proportional to the size of the ganglion cell layer.

These differences between rat and man are related to the preferential adaptation of the rat retina for nocturnal vision.

26. Distribution of native and foreign albumin-containing cells in prenatal rat forebrain. By MARION E. CAVANAGH and ANNE WARREN. *Department of Physiology, University College London, London*

Previous experiments showed that foreign proteins, sheep serum, could be detected immunologically in ventricular zone cells of newborn rat forebrain after injection into the lateral ventricle (Cavanagh & Warren, *J. Physiol.* **353**, 1984). These experiments have now been extended by examining the distributions of sheep albumin after the injection of sheep serum proteins into the ventricles of rats at days 16E, 18E and 20E and comparing these with the distributions of naturally occurring rat albumin within the cells of the developing forebrain. Time-mated pregnant rats were anaesthetised with sodium pentobarbitone. After exposure of the uterus the pups were injected with fetal sheep serum into the lateral ventricle through the uterine wall. At 16E the uterus was opened and some pups were injected through the amnion only. After various time intervals the brains of both injected and control pups were fixed in Bouin's fluid. Sheep and rat albumins were separately detected on adjacent paraffin sections using PAP-immunocytochemistry. Pups from a separate series of time-mated animals were fixed at daily intervals from day 14E to day 21E and stained for rat albumin. At 14E although albumin could be detected in the CSF in the ventricle it was not present in any cells of the forebrain. Thereafter it began to be increasingly detected in cells of the ventricular zone until at 21E it was seen as a thick band of positive cells. At day 17E it was first detected in cells of the mesocortical plate and at day 18E in cells of the isocortical plate. The pattern of distribution of the injected sheep albumin was, at all three ages, the same as that of the rat albumin on the adjacent section. These results tend to suggest that the presence of albumin-containing cells within the developing rat brain can be accounted for by uptake.

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27. Effects of the anti-tumour drug taxol on developing neurons in explants of CNS tissue. By MARGARET M. BIRD. *Institute of Anatomy B, University of Aarhus, Denmark and Department of Anatomy, London Hospital Medical College (Fig. 2A–D)*

Taxol, isolated from the plant *Taxus brevifolia*, is a powerful inhibitor of mitosis and has been used as an experimental anti-tumour drug. Cells exposed to taxol display increased numbers and abnormal groupings of microtubules.

Explants of optic tectum from chick embryos of 9 or 10 days were cultured for 2–3 weeks after which taxol (5 μ m) was added to the culture medium. After further periods of culture ranging from 1 day to 21 days the taxol-treated and control explants were fixed and prepared for EM observation.

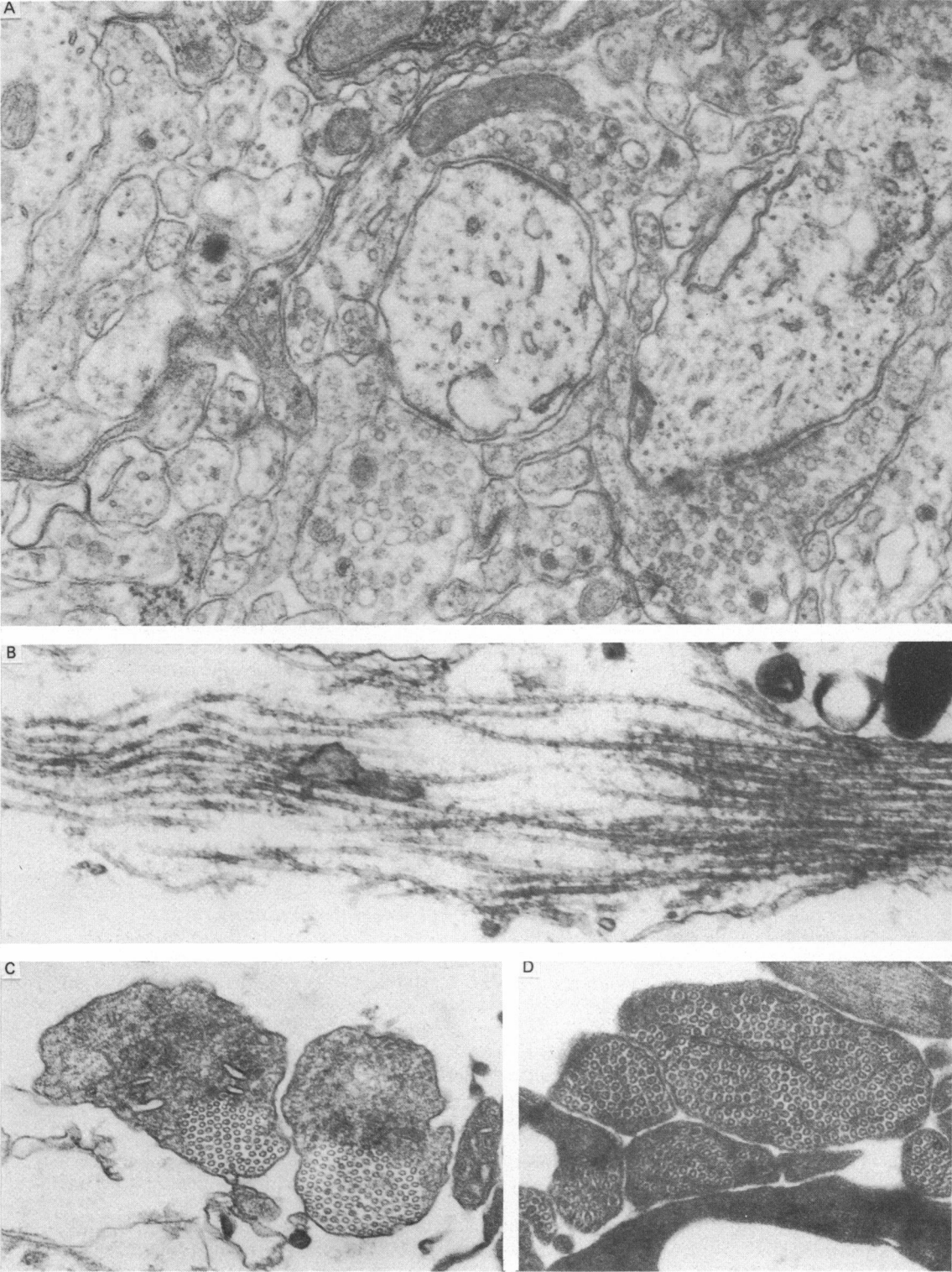


Fig. 2

Neuronal somata and processes in control cultures (Fig. 2A) displayed a normal pattern of cytoskeletal elements. In treated explants, microtubules were greatly increased in number, often in large bundles, and displayed various abnormal associations with cytoskeletal elements and with cell and organelle membranes (Fig. 2B-D). Microtubules in bundles were frequently associated with neurofilament-like and other finer filamentous material disposed in a hexagonal lattice around the parallel microtubules. Microtubules were also induced to form striking concentric ring formations, each ring separated from the next by a sheet of electron-dense material, and to align in rows beneath the plasma membrane or around mitochondria or smooth endoplasmic reticulum (Fig. 2D). Although individual microtubules were generally normal in appearance, central dense cores, side-arms or cross bridges linking microtubules to one another or to membranous elements, incomplete microtubules and microtubule doublets were all seen more frequently than in control tissue. Increases in number or other microtubule abnormalities were not seen in all neural profiles nor throughout individual profiles, suggesting that not all microtubules are equally susceptible to the effects of taxol and that taxol may therefore be useful in studies of microtubule heterogeneity in neurons. The effects of taxol on microtubules also suggests that it might be interesting to study axonal transport in taxol-treated neural tissue.

28. Three dimensional reconstruction of arteriovenous anastomoses (AVAs) in rat interscapular brown adipose tissue (ISBAT). By J. O. NNODIM, J. D. LEVER and D. SYMONS. *Department of Anatomy, University College, Cardiff*

In a previous communication (Lever *et al.* *J. Anat.* 139, 1984) the presence of arteriovenous connections at peripheral and perilobular sites in rat ISBAT was inferred from a study of Van Gieson-stained serial sections of the tissue after topical intra-arterial and intravenous India ink injection.

By means of a microreconstruction technique three dimensional models of AVAs in rat ISBAT have been obtained. Heparinised young adult rats were used in this investigation. Following India ink injection either into the subclavian artery or azygos vein, ISBAT pads were removed by wide dissection, formol-fixed and paraffin-embedded. Osmicated, paraffin-impregnated, straight segments of sciatic nerve were included at opposite corners of each paraffin block and served as reference markers. All blocked material was serially cut (at 13 μm), mounted and stained by the Van Gieson technique. After microscopic identification and photomicrography of AVAs, relevant sections were projected at standard magnification and relevant profiles, including reference markers, were traced. Tracings were transferred onto modelling wax sheets (Detrey) which were stacked with reference marks in alignment so as to ensure accurate superimposition. During the sculpting of individual components, representative of vascular entities, wax plates were accurately superimposed and secured by surface treatment with Araldite adhesive. Arteries, veins and AVAs were finally differentiated in the model by the use of coloured paints as a final covering.

29. Quantitative changes in the microvasculature of the rat coeliac-superior mesenteric ganglion (CSMG) with age. By D. M. BAKER and R. M. SANTER *Department of Anatomy, University College, Cardiff*

Several morphometric studies on the effects of age on the vasculature of various parts of the CNS have been made. As yet no comparable quantitative information exists to describe age associated changes in autonomic ganglia. This communication reports on the age differences in the microvasculature of the rat CSMG.

Four groups of 10 albino rats of 6, 12, 18 and 24 months were whole body perfused with a glutaraldehyde/formaldehyde/PVP mixture in cacodylate buffer. Perfusion fixation of the animals ensured ideal conditions for automatic image analysis. Following fixation, ganglia were removed, diced, osmicated, ethanol dehydrated and embedded in Spurr resin. Two blocks from each animal were randomly selected and from each, 2 μm sections were cut. Every eighth section was saved until 30 sections per block had been obtained. From every other section of those saved a field of $1.1 \times 10^5 \mu\text{m}^2$ was randomly selected for quantitative analysis of the vasculature using a Quantimet 800 image analyser. For each ganglion, total vascular profile area and perimeter were obtained and expressed as a ratio of the frame area. By invoking Delesse's principle these

ratios are those of vascular volume to ganglion volume (V/V) and vascular surface area to ganglion volume (SA/V) respectively. Thus comparing different age groups gives an indication of age changes in whole ganglion vascular volume and surface area. For both, a significant decline in the ratio was observed with age. Using Fischer's one way analysis of variance for V/V, $F = 5.23$ and for SA/V, $F = 11.23$. This indicates a reduction in the blood supply to the ganglion, probably due to a fall in individual vessel size, reducing both lumen volume and wall surface area, rather than a reduction in the number of vessels. This decline with age was not gradual, a dramatic reduction occurring between 12 and 18 months.

The significance of these findings to the ageing of autonomic ganglia was discussed.

30. The influence of insulin therapy on myelinated nerve fibre size and skeletal growth in experimental diabetes. By I. G. M. DUGUID (introduced by A. K. SHARMA). *Department of Anatomy, University of Aberdeen, Marischal College, Aberdeen*

Myelinated nerve fibre size and skeletal growth are known to be less in streptozotocin-induced diabetic rats compared with age-matched controls. In a previous study, 8 weeks conventional insulin treatment normalised body weight, improved skeletal growth but did not ameliorate nerve fibre size in the tibial nerve (Sharma *et al. Neuropathol. Appl. Neurobiol.* **10**, 1984). It was considered important to establish whether insulin treatment over a longer duration is beneficial to nerve fibre maturation.

Four groups of male Sprague-Dawley rats, aged 11 weeks and weighing approximately 450 g, were studied over 4 months: onset controls, end controls, diabetics, and diabetics injected daily with ultralente insulin (Novo-Alle.) The insulin dose was adjusted daily according to blood glucose estimations using a Glucometer (Ames). Satisfactory control of blood glucose and glycosylated haemoglobin (HbA_{1c}) was achieved throughout the study.

Body weight decreased progressively in the untreated diabetics whilst that of the insulin-treated animals increased in parallel with the end controls. Tibial length in the insulin-treated group did not differ from that of the end controls, and both were significantly greater than the untreated diabetics and the onset controls ($P < 0.001$); there was no difference between the latter two groups. Thus, skeletal length was normalised by insulin treatment. Myelinated fibre diameter increased between the onset and end controls ($P < 0.01$). In the untreated diabetic rats fibre diameter was not only significantly less than the end controls ($P < 0.001$) but also less than the onset controls ($P < 0.01$). Fibre diameter in the insulin-treated group was significantly less than that of the end controls ($P < 0.01$) and although it did not differ from the onset controls it was significantly greater than the untreated diabetics ($P < 0.05$). Therefore, fibre size was not normalised but did appear to be improved compared with the untreated diabetics.

31. Preliminary observations on the effects of peripheral nerve conditioned medium on neurite outgrowth from mouse spinal cord slices. By S. CORNISH, P. N. ANDERSON*, J. MITCHELL and D. MAYOR. *Human Morphology, University of Southampton, and *Department of Anatomy and Biology as Applied to Medicine, The Middlesex Hospital Medical School, London*

Regeneration of transected axons in the CNS can be stimulated in the environment of a peripheral nerve implant. It has been suggested that peripheral nerve derived neuronotrophic factors may be responsible for this phenomenon.

In this study the effects of a number of mouse sciatic nerve and spinal cord conditioned media (CMs) on the outgrowth of neurites from slices of neonatal mouse spinal cord were investigated. All the CMs tested produced a significant ($P < 0.01$) increase in the mean number and length of neurites extending from the slices. Preincubation of the sciatic nerve CM on polyornithine-coated dishes resulted in a significant ($P < 0.005$) decrease in its ability to stimulate neurite outgrowth. It has been proposed that peripheral polyornithine-binding neurite promoting factors act mainly on axons which normally extend outside the CNS (Varon *et al. Prog. Clin. Biol. Res.* **79**, 1982). In the case of the spinal cord such axons should be the processes of motor neurons. Spinal cord slices were, therefore, divided into dorsal and ventral halves before testing their response to sciatic nerve CM.

In control cultures neurite outgrowth from ventral halves was greater than that from dorsal halves. In experiments using CM only the ventral halves showed a significant ($P < 0.001$) increase in neurite outgrowth.

A histological analysis of the spinal explants was performed using an IBAS image analyser. After 5 hours of culture, a high percentage of neurons had become necrotic, and by 24 hours the remaining neurons were significantly smaller ($P < 0.001$). However, the size of the neurons in the cultured slices subsequently gradually increased. By 48 hours the size of neurons cultured in sciatic nerve CM was increased compared with neurons cultured in control medium.

Thus the CNS neurite promoting activity of peripheral nerve CM binds to polyornithine, affects ventral spinal cord neurons preferentially and increases the size of cultured neurons. The significance of these findings was discussed.

This project, the Ferndown Project, was supported by the International Spinal Research Trust.

32. Further evidence for neurotropism. By P. N. ANDERSON and M. TURMAINE, *Department of Anatomy and Biology as Applied to Medicine, The Middlesex Hospital Medical School, London*

In a classic study of neurotropism Weiss and Taylor (*J. Exptl. Zool.* **95**, 1944) used grafts of abdominal aorta and its bifurcation as a Y-tube for regenerating rat sciatic nerve axons to grow through. Regenerating axons were apparently not attracted to a distal nerve stump inserted into one of the common iliac arteries.

In the present study adult female CBA mice were anaesthetised with ether and their left common peroneal nerve cut. The proximal stump was sutured into the lumen of an aorta graft from another CBA mouse and the distal nerve sutured into one common iliac artery. The animals were killed 1, 2, 4, 6 or 8 weeks later by perfusion with fixative through the left ventricle under deep anaesthesia. Of the grafts 1 mm segments were processed into Araldite. Transverse sections of the segments were examined using light and electron microscopy.

One week after operation the elastic laminae of arterial grafts remained intact, and the smooth muscle cells in the walls of the graft remained alive, but the endothelium was no longer present. There was little evidence of axonal regeneration in the aorta, which was largely filled with amorphous material. Four weeks after operation the arterial wall was unchanged, but the lumen was occupied throughout by many blood vessels and a variety of cell types. Axonal regeneration was, however, limited to about 2 mm. By six weeks after operation regenerating axons had entered the distal nerve (4–6 mm from the proximal stump) and in some cases many myelinated axons were present. Within the aorta grafts the axons were found in perineurium-bounded fascicles. Very few axons were identified near the distal end of the common iliac artery which did not contain the distal nerve. Similar experiments using aorta grafts from another strain of mice were also described.

These results are similar to those of Politis *et al.* (*Brain Research*, **253**, 1982) who used silastic Y-tubes. The mechanisms by which distal nerve stumps attract regenerating axons were discussed.

33. The fate of radiolabelled epididymal spermatozoa after artificial insemination. By P. J. MARSH and J. M. MCLEAN. *Department of Anatomy, University of Manchester*

Internal fertilization involves the introduction of millions of spermatozoa, suspended in a complex fluid medium, into the female reproductive tract. Most of the surplus spermatozoa not involved in fertilization are rapidly removed from the reproductive tract, but some may gain access to the female tissues. In an outbred population this is potentially hazardous to the female and her conceptus since it involves an antigenic challenge to the maternal organism. This work was an attempt to establish whether radio-labelled spermatozoa, introduced artificially into the female reproductive tract, are absorbed, and, if so, in which tissues they accumulate.

The reproductive tract, iliac and popliteal lymph nodes, and samples of blood, liver, spleen, thymus, lung, gut and muscle from thirty DA female rats were examined for radioactivity following artificial insemination of Indium-III-oxine labelled, allogeneic (AO), epididymal spermatozoa. The DA females, in groups of five animals, were killed 4, 8, 16, 24, 48 and 72 hours after insemination.

After the insemination of Indium-labelled spermatozoa, most of the activity taken up was absorbed during the first 16 hours. The blood provided the main route for uptake of label, and

the uterine afferent lymphatics contributed the rest. After its initial peak at 4 hours, the blood level of radioactivity decreased significantly throughout the observation period, while that of the iliac lymph nodes, liver, spleen and thymus increased significantly. There were no significant changes in the levels of radioactivity in the lung, gut, muscle or popliteal nodes. The radioactivity was assumed to represent spermatozoa or spermatozoal fragments. These observations gave no information concerning the cellular events taking place in these tissues, but the accumulation of label within them probably represents an antigenic challenge.

34. Granulated metrial gland cells in the non-traumatised uterus of mice with deciduomata. By I. J. STEWART. *Human Morphology, University of Southampton*

Granulated metrial gland (GMG) cells differentiate in small numbers throughout the endometrium of mice sensitised to respond to a decidual stimulus. If this stimulus is not given the GMG cells soon disappear.

Ovariectomised mice were primed with oestrogen, maintained on progesterone and a silk thread was inserted into part of the lumen of the left uterine horn at a stage equivalent to day 3 of pseudopregnancy. Normal deciduomata developed and these were restricted to the area around the silk thread. Numerous GMG cells differentiated in the mesometrial decidua and a metrial gland was also formed. By 3 days after insertion of the thread there were no GMG cells in the non-traumatised right uterine horn. However GMG cells were still found in the non-traumatised part of the left uterine horn. These GMG cells are present until at least 5 days after they are lost from the right uterine horn. There did not appear to be any change in the numbers of GMG cells in the non-traumatised left uterine horn during this time. Mitotic activity in stromal cells was determined in colchicine treated animals killed 3 days after uterine trauma and showed that activity was high in the non-traumatised left uterine horn but was very low, or absent, in the right uterine horn. The association of this mitotic activity with the maintenance of GMG cells in the left uterine horn was discussed in relation to the possibility that stromal/decidual cells have an influence on the differentiation of GMG cells from their bone marrow precursors.

35. The morphology of cultured mouse endometrial cells. By D. D. MUKHTAR and I. J. STEWART. *Human Morphology, University of Southampton*

The granulated metrial gland cells which are found in the pregnant mouse uterus have been shown to differentiate from cells originating in the bone marrow but the mechanisms which control their differentiation are unknown. However, *in vivo* experiments have indicated that factors involved in this process may be released by developing decidual cells. As a preliminary to investigating the factors involved in the differentiation of granulated metrial gland cells *in vitro*, we have studied the morphology of mouse endometrial cells, including decidual cells, grown in culture for up to 10 days.

Cell suspensions of endometrium were prepared from virgin uteri and deciduomata at days 5 and 8 of pseudopregnancy and maintained in MEM. In 1 day cultures from virgin mice the majority of cells were irregular in outline with many cytoplasmic processes and probably represent the endometrial stromal cells. Groups of epithelial cells were present and a third population consisted of small round cells with a round or kidney shaped nucleus. These three cell types were also present in 1 day cultures of cells from deciduomata but in addition two groups of large cells were identified. One group was rounded in shape, the other had an irregular outline but both were believed to be decidual cells. The large cells were more numerous in cultures from day 8 than from day 5 deciduomata. All the populations were maintained throughout the period of culture although some changes in morphology occurred. The stromal and decidual cells in particular showed considerable growth. The effects of progesterone supplements on the cultured cells and of growing the cells in collagen coated dishes were also studied. The relationship between the cells maintained *in vitro* to those present *in vivo* was discussed.

36. A comparison of the invasion of endovascular trophoblast in rats and mice. By J. SKIDMORE (introduced by S. PEEL). *Human Morphology, University of Southampton*

The extent of the invasion of endovascular trophoblast has been studied in rats and mice killed between day 8 and 19 of pregnancy. Particular attention has been paid to the arrangement of the vessels passing from the metrial gland to the placenta by examining serial sections, some of which have been used to prepare three dimensional reconstructions. By day 12 of pregnancy in the rat, endovascular trophoblast lined vessels in the decidua basalis, but none was present in vessels in the developing metrial gland. Rapid invasion resulted in endovascular trophoblast extending 1–2 mm into metrial gland vessels at day 13 and 14. By day 16 the endovascular trophoblast had reached the periphery of the metrial gland and in some specimens at day 16 and 17 trophoblast was seen in vessels at the base of the mesometrium itself. The reconstructions indicated that trophoblast invades blood vessels as a continuous tongue of tissue: endovascular trophoblast was not seen lying freely in the lumina of vessels. Granulated metrial gland cells were found around all vessels in the metrial gland which contained trophoblast but there did not appear to be any difference in the numbers of granulated cells surrounding vessels containing trophoblast and those which did not. The endovascular trophoblast was associated with deposits of diastase-fast periodic acid–Schiff-positive fibrinoid material. The extensive endovascular trophoblast invasion seen in vessels in the decidua basalis of the rat did not occur in the mouse and there was no invasion into the metrial gland.

37. The association between granulated metrial gland cells and trophoblast in the rat. By S. PEEL. *Human Morphology, University of Southampton*

Granulated metrial gland (GMG) cells occur in large numbers in the decidua basalis and metrial gland of rats and mice and many degenerate *in situ* as pregnancy proceeds. In the mouse, some GMG cells apparently migrate and degenerate in the placental labyrinth. Their association with degeneration of adjacent labyrinthine trophoblast has led to the suggestion that this may be a site of functional significance. A survey has therefore been made of the relationship between GMG cells and labyrinthine, and other trophoblast, in rats between day 10 and day 20 of pregnancy.

At day 10, although the invasion of decidua basalis by the ectoplacental cone brought many trophoblast and GMG cells into close association, the majority of cells in the region appeared normal. GMG cells were found in the labyrinth from day 14 but they were not as apparent as GMG cells in the mouse labyrinth: even at day 18 of pregnancy in the rat, when the GMG cells in the labyrinth were relatively numerous, degenerative changes were not a prominent feature.

Trophoblastic invasion of metrial gland blood vessels from about day 14 resulted in a close association between GMG cells and endovascular trophoblast. In some regions fibrinoid separated the two cell types but apparently normal GMG cells were found, adjacent to the lumen, lying between endovascular trophoblast cells. From about day 16 glycogen cells, presumed to be of fetal origin, were seen surrounding the metrial gland vessels which were lined with endovascular trophoblast. Similar glycogen cells were observed which had apparently migrated so that during the last few days of pregnancy they were present around vessels which were not lined by endovascular trophoblast. Although numerous GMG cells lying adjacent to the glycogen cells in the metrial gland have been examined, no morphological evidence of a functional interaction has been observed.

38. The distribution of leucocyte surface antigens in metrial gland tissue of deciduomata and of ovariectomised pregnant rats. By B. S. MITCHELL (introduced by S. PEEL). *Human Morphology, University of Southampton*

The rat metrial gland is populated by a variety of cells, some expressing leucocyte common (LC), Ia or Thy 1.1 antigens. To determine whether there is a role for paternal antigens and ovarian hormones in the induction of these cells, metrial glands from deciduomata-bearing rats at days 10 and 13 of pseudopregnancy and primigravid rats at day 15, ovariectomised at day 12, have been examined. The results have been compared with metrial glands from primigravid rats at the appropriate stages of gestation. Distribution of leucocyte surface antigens was determined

in cryostat sections and percentages of labelled cells in smears of single cell preparations using immunofluorescence. Percentages of granulated metrial gland (GMG) cells were determined in paraffin sections reacted for glycoprotein.

LC antigen was expressed on fewer cells from decidualata at day 13 of pseudopregnancy ($7.0 \pm 0.3\%$) than from controls ($8.9 \pm 0.5\%$) ($P < 0.02$) and on more cells from ovariectomised primigravid rats at day 15 ($15.6 \pm 1.5\%$) than from controls ($9.3 \pm 0.05\%$) ($P < 0.01$). No other significant differences in percentages of antigen-bearing cells, or in percentages of GMG cells were detected. Distribution of LC, Ia and Thy 1.1 antigens in metrial glands from decidualata-bearing and ovariectomised rats was similar to their distribution in tissue from primigravid pregnancy except that clusters of Thy 1.1 antigen-positive cells were absent from tissue of ovariectomised rats.

As the majority of LC antigen-positive cells in primigravid pregnancy are GMG cells, these observations indicate that ovariectomy is related to an accumulation of LC antigen-positive cells which are not GMG cells. The alteration in the proportion of GMG cells and LC antigen-positive cells in decidualata may mean that some GMG cells in decidualata do not express LC antigen. Alternatively it may be that fewer LC antigen-bearing bone marrow precursors for GMG cells were present in the glands from decidualata than in glands from controls. Examination of routine histological sections does not reveal obvious differences between metrial glands from decidualata and from primigravid pregnancy which can be related to the differences in surface antigens.

39. Receptor mediated endocytosis of IgG by human placental coated vesicles and the process of concentrating ligand in selective transepithelial transport of protein. By C. D. OCKLEFORD and L. DEARDEN. *Department of Anatomy, University of Leicester Medical School, University Road, Leicester*

An important function of absorptive epithelia is to divide compartments where proteins are present at different concentrations. In the case of the human placental syncytiotrophoblast epithelium it has been shown that fetal (cord) blood may contain higher concentrations of maternally derived IgG than the mother's own blood. Theoretically this difference might be explained by concentration during uptake or differences in rates of degradation in the fetal and maternal compartment.

It is shown by a simple calculation that the concentration of IgG in the lumen of placental coated vesicles (most of which are present at the apical surface of the syncytiotrophoblast) is higher than the concentration in maternal blood. The average concentration of IgG in the coated vesicle lumens was 44 mg/ml whereas the concentration of IgG in maternal serum is 8–16 mg/ml.

This finding indicates that receptor mediated endocytosis, as well as being selective, is a *concentrative* process which may at least in part account for the raised concentration of maternal IgG in the fetal blood.

40. Intervillous bridges in the human placenta at term. By G. J. BURTON. *Department of Anatomy, University of Cambridge, Cambridge*

Intervillous bridges have long been reported to be a common feature of the mature human placenta. It is thought they are created by fusion of the syncytium of closely proximated villi, and may act as an internal strut system providing support for the villi (Jones *et al. J. Anat.* **124**, 1977). More recently Kustermann (*Anat. Anz.* **150**, 1981) claimed these structures to be artifacts, caused by tangential sectioning through the syncytium. In order to determine the nature of these alleged bridges, 250 examples were followed through serial sections.

Material was obtained from three normal healthy placentae delivered by Caesarian section at term. Following fixation in 1% glutaraldehyde, 1% formaldehyde in 0.1M cacodylate buffer, the tissue was osmicated, dehydrated and embedded in Spurr resin. Sections were cut at 0.2 μ m, and every tenth section mounted and stained with methylene blue for light microscopy. Between 60–100 such sections were prepared for each placenta.

All the putative intervillous bridges seen could be followed through in their entirety at high resolution. It was thus possible to classify them into four types according to their origin: Type 1 (20%), section through area of syncytial fusion or 'true bridge'. Type 2 (53%), tangential

section through the syncytium filling the crotch of a villus branching point. Type 3 (4%), tangential section through the syncytium lining the angle of a tight 'U' bend of a villus. Type 4 (23%), tangential section through the syncytium of a sinuous villus.

It is clear therefore that whilst areas of true syncytial fusion between adjacent villi do occur, the majority of supposed intervillous bridges seen in sections are indeed artifacts. Furthermore, it is frequently impossible to predict from examination of only one section whether a particular intervillous bridge is genuine or artifactual. Only by following the bridge through the preceding and succeeding sections can its true nature be determined.

This work was supported by a grant from Action Research – The National Fund for Research into Crippling Diseases.

41. The effects of anti-visceral yolk sac antisera on rat embryos and visceral yolk sacs grown in culture. By S. K. WEBBER (introduced by F. BECK). *Department of Anatomy, University of Leicester Medical School, Leicester*

It has frequently been reported that exposure of rat embryos to certain tissue antisera causes embryonic malformation and retardation. Recent papers have suggested that this is due to decreased pinocytosis in the visceral yolk sac (VYS), a process vital to embryonic nutrition (Freeman *et al.* *J. Embryol. exp. Morph.* **71**, 1982). Ultrastructural evidence bearing upon these reports was sought.

Rat embryos 9½ days old were explanted and cultured by the New roller culture method, using homologous serum as the culture medium (New, *Biol. Rev.* **53**, 1978) and to this was added small amounts of antiserum prepared against rat VYS. After 2 days in culture the embryos were morphologically assessed and samples of VYS were taken for electron microscopy.

The antiserum was found to be highly teratogenic. Stereological analysis of the VYS cells showed that the addition of antiserum to the culture medium decreased the endocytic compartment of the cells by approximately two thirds of the control value. Details of this and other ultrastructural changes seen in the cells were recorded.

Antisera were raised against whole rat VYS; these were also found to be highly teratogenic. A dose-response experiment was performed and a clear relationship was demonstrated between dose of antiserum applied, malformations produced and severity of effect on the VYS cells. The results of these experiments correlated well with the findings of Freeman, Brent and Lloyd. They support the hypothesis that teratogenic antisera cause a dysfunction in the VYS which leads to a nutritive supply insufficient to support the normal growth and development of the embryo.

This work was supported by a grant from the MRC.

42. Changes in the human hair pattern. By LYN I. L. JONES (introduced by A. J. PALFREY). *Department of Anatomy, Charing Cross and Westminster Medical School, London*

Hair shaft diameter and follicle density are two of the many variables which determine the human hair pattern. Gross studies of this pattern have been published, but the nature of the changes which occur at puberty has not been documented. One noticeable change, the replacement of the fine 'vellus' hair by coarse 'terminal' hair is relevant to the study of both common baldness and female hirsutism.

In this study certain measurements were made of the hair pattern on the lower leg of male subjects. Five adults (mean age 21.8 years), 5 adolescents (13.4 years) and 5 boys (7.9 years) were studied. The terminal hair pattern in 3 adult females (21.8 years) was also observed. The changes in each measurement with age were determined and comparisons made between the various measurements.

The results showed that hair shaft diameter, the density of terminal hairs, the rate of hair growth, the proportion of hairs in the active growth phase, and the proportion of terminal hairs in groups of three all increased with age. The differences in each of these measurements (except for the proportion in the active growth phase) were greater between the adolescents and the adults than between the boys and the adolescents. Shaft diameter and the rate of growth varied

in a similar manner within each group of subjects, suggesting a common cause for the age associated changes in these measurements.

The grouping pattern of the terminal hairs in the adult females resembled that in the boys, but differed from that in the adult men. This observation suggests that the number of terminal hairs growing in a group of follicles is more dependent on the systemic androgen level than on the inherent maturation of the follicle.

43. An X-ray microanalysis study of the intracellular elemental concentrations of thymocytes in tissue sections and in cells in suspension. By ALICE WARLEY, I. W. MORRIS and MARION D. KENDALL. *Department of Anatomy, St Thomas's Hospital Medical School, London*

The nuclei and cytoplasm of frozen, freeze-dried sections of mouse thymus and suspensions of thymus cells were analysed by X-ray microanalysis using a Link 860 system and an AEI EMMA-4. From each of nine animals, at least 20 sections of cells were studied (each 0.1–0.5 μm mass thickness). The conditions of analysis were: a spot size of 1 cm diameter at 16000 magnification, 100s live time, 60 kV accelerating voltage and 4 nA beam current. Spectra were analysed with Link's Quantem program for peak deconvolution and background subtraction. The levels of Na, Mg, P, S, Cl, K, Fe, Zn and Ca were determined by reference to previously prepared and analysed standards of known molar concentrations.

In all cells, the nuclei contained more P and S than the cytoplasm ($P < 0.001$) and there were other small differences between the two compartments. The concentrations of the readily diffusible elements such as Na and Cl were greatly elevated in the cells in suspension, perhaps due to the separation techniques; most of the other elements maintained constant levels or were only slightly elevated.

The results show that simple cell separation procedures, as used in many immunological techniques, may not yield cells that are typical of similar cells *in vivo*.

44. Thymic nurse cells and types 2 and 3 epithelial reticular cells. By MARION D. KENDALL. *Department of Anatomy, St Thomas's Hospital Medical School, London*

Thymic nurse cells have been identified and studied mainly *in vitro*. They are large cells with the capacity to hold on average 80 thymocytes (although up to 2000 have been recorded) within their cytoplasm by emperipolesis. The enclosed thymocytes form a minor subpopulation of the cortical thymocytes (probably 0.5–1 % of the total), and the cells are of variable phenotype. Single thymic nurse cells have been convincingly demonstrated in the thymus *in vivo* by electron microscopy and it has been suggested that they are part of the population of types 2 and 3 epithelial reticular cells of the cortex.

Histological and electrophysiological evidence is presented here to suggest that the types 2 and 3 epithelial reticular cells of the thymus are all thymic nurse cells and that they are arranged in a more zonal manner than was previously recognised. This zone forms a distinct microenvironment through which any cell that matures from prothymocyte to typical cortical thymocyte would have to pass. Because of the short cell cycle times in the thymus, passage could be rapid, and this could account for the fact that thymocytes in thymic nurse cells appear to represent only a minor part of the total thymus lymphocyte population.

45. Structural characteristics of the luminal surface of human ileum. By P. A. SANFORD, M. TURMAINE*, C. B. WILLIAMS†, and N. W. WONG‡ (introduced by B. A. WOOD). **Department of Physiology*, †*Department of Anatomy and Biology as Applied to Medicine, The Middlesex Hospital Medical School*, ‡*Department of Endoscopy, St Mark's Hospital and ‡Department of Gastroenterology, The Middlesex Hospital, London* (Fig. 3)

Electron micrographs of rat distal ileum showed filamentous bacteria embedded in brush borders of the villous epithelia. Such micro-organisms could not be detected in fasted animals (Gould & Sanford, *J. Physiol.* **350**, 1984). To extend these studies human distal ileum was investigated. On fourteen occasions when patients were subjected to colonoscopy the ileum was examined. Samples were taken and rapidly immersed in 1/4 strength Karnovsky fixative at pH 7.4. Sections were prepared for transmission electron microscopy.

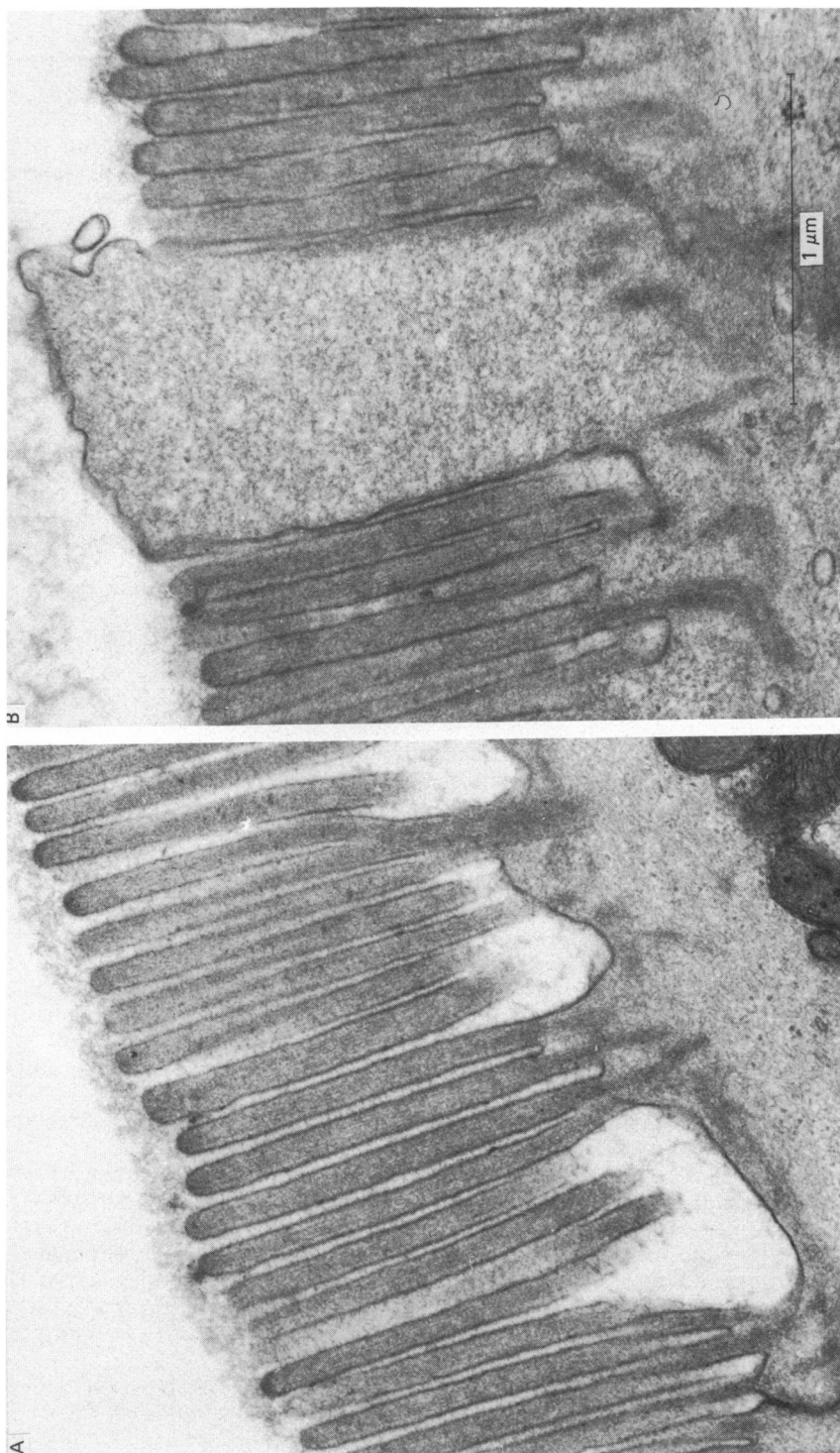


Fig. 3

None of the bacteria detected corresponded to those recorded in rat ileum. Two characteristics, however, deserve consideration. The first, frequently encountered on the villus, involved several microvilli sharing a common base and giving a tufted appearance (Fig. 3 A). These groups were separated by relatively straight stretches of plasma membrane. A similar finding has been reported in the jejunum of infants with marasmus (Brunser *et al. Gastroenterology* 70, 1976). The second finding was of regions where the brush border had failed to develop (Fig. 3 B). It was as if several microvilli had fused together to form a clump. Few microfilaments could be detected in these structures which have only been observed in seven samples. They varied from frequent in a patient with carcinoma of the caecum to occasional in patients with villous adenoma, ulcerative colitis and colonic polyps.

These clumps do not appear to have been described previously. Their significance and that of the tufted microvilli is unknown. However, it is interesting that clumps were detected where intestinal carcinoma has been diagnosed or situations exist associated with a greater incidence of subsequent neoplasm development. Might a study of ileal ultrastructure provide an indication of later colonic abnormalities? Certainly an exciting possibility for those who study ultrastructural characteristics is the potential identification of preneoplastic changes in the mucosa which is to casual inspection histologically normal.

46. A role for macrophages in normal developing muscle. By ENAM A. ABOOD & MARILYN M. JONES. *Department of Anatomy, St Thomas's Hospital Medical School, London*

Muscles from rats of various ages were examined for the presence of macrophages. A role for these cells has not previously been described in developing skeletal muscle.

Samples of the diaphragm, rectus abdominis and calf muscles were removed from fetal growing and adult CSE rats (two animals of each of the following ages: 14 days, 15 days, 16 days, 18 days, and 20 days gestation, newborn, 1 day old, 3 days old, 1 week, 2 weeks, 4 weeks, 8 weeks and 14 weeks old), and examined for acid phosphatase activity using light microscopy. In addition, the diaphragm was examined by means of electron microscopy.

The number of macrophages was numerous in the young animals and declined with age.

Macrophages were found in close association with muscle fibres, particularly in the fetal and newborn animals, and some included myofibrils at different stages of phagocytosis.

The conclusion that macrophages may play a role in skeletal muscle development was discussed.

47. Sex determination from the pelvis in a Dutch skeletal series. By SUSAN M. MACLAUGHLIN and MARGARET F. BRUCE. *Department of Anatomy, University of Aberdeen*

The pelvis is regarded as the most reliable skeletal indicator of sex. Morphological differences are frequently used to distinguish male from female type pelvises. However, objections to the morphological approach include (a) the essentially subjective nature of the method, (b) its dependence on the experience of the observer and (c) it is less readily amenable to statistical analysis.

As a result, there has been an increasing tendency towards a more objective morphometric approach. One type of morphometric analysis involves the use of indices.

The present study describes and tests the discriminatory capacity of a pelvic index based on a comparison of pubic length and acetabular diameter. The index was tested on a skeletal series ($N = 133$) of documented sex housed in the University of Leiden. The mean male index (1.12 ± 0.08 ; $N = 70$) and the mean female index (1.37 ± 0.14 ; $N = 63$) were determined. The mid-point between these means (1.24) was used as a sectioning point to test the discriminatory power of the index. Values higher than the sectioning point indicated female sex and values lower than the sectioning point indicated male sex. The results showed a 92.5% accuracy of sex determination.

The advantages and limitations of this pelvic index were discussed and compared with other techniques in current use.

48. Which cnemic index? By JULIET F. CROSS and MARGARET BRUCE. *Department of Anatomy, University of Aberdeen*

The shape of the proximal tibial shaft is usually described and quantified by means of the cnemic index (CI). This gives an indication of the extent of mediolateral flattening of the shaft and is calculated thus:

$$\frac{\text{maximum anteroposterior diameter}}{\text{mediolateral diameter}} \times 100.$$

These measurements are taken conventionally at the level of the distal margin of the nutrient foramen. Andermann (*Am. J. Phys. Anthropol.* **44**, 1976) suggested that, because of the variability in position of the foramen, the cnemic index should be taken at an osteometric landmark – at one third of the tibial shaft length from the proximal extremity. He found substantial differences in bone shape, as expressed by the two cnemic indices, at these levels in an Amer-Indian skeletal series.

Bone shape at these two levels was compared by means of the respective cnemic indices in a Scottish mediaeval population. There was no significant difference in the extent of mediolateral flattening of the shaft as expressed by the indices at the nutrient foramen level compared with the one third level. The mean level of the nutrient foramen along the tibial shaft was some 68 % from the distal end, i.e. it lay somewhat more proximal, but very close to, the one third level. In Andermann's study the mean nutrient foramen level was at some distance from, and even more proximal to, the one third level.

Thus it is suggested that if the CI (nutrient foramen) is to provide a meaningful measure of tibial shaft shape, the level of the nutrient foramen along the shaft must also be known. It is further suggested that differences in the relative position of the nutrient foramen may reflect differences in the growth rates of the tibia in different populations.

49. Developments in techniques for the measurement of cranial shape in the hominoids. By P. O'HIGGINS. *Department of Anatomy, The University of Leeds*

The last few decades have seen the introduction of multivariate techniques into the study of Hominoid taxonomy. The variates subjected to analysis have traditionally comprised linear and angular measurements. However, these methods of measurement suffer from several drawbacks. Principal amongst these are problems associated with defining homologous landmarks, and of characterising the whole shape of complex and irregular structures. It is often impossible to reconstruct the shape of the bone under consideration from measurements of the type generated by traditional osteometry.

An ideal system should be independent of homologous points and the observed measurements should be embedded in a mathematical function which:

1. accurately measures the form or any part of it.
2. allows controls for the allometric correction of size dependent shape differences.
3. produces data which are suitable for multivariate compression and analysis to allow comparison of shapes.
4. allows the reconstruction of the original form from the variate set which is to be analysed.

The present study has compared several techniques for the measurement of cranial shape in order to compare their efficacy in characterising the shape of mid-sagittal sections of the crania of various Hominoid groups. One of these is the technique of Fourier analysis which goes some way to conforming with the above criteria. When the Fourier coefficients derived from analysis of these outlines are compounded by multivariate techniques, similarities and differences between various groups emerge. The pattern of discrimination is quite similar to that derived from multivariate combination of cranial dimensions and angles but the extent of separation is much more marked and allows a higher degree of taxonomic certainty in classifying unknown forms. The application of this method to the study of three dimensional shapes is being developed.

50. The extensor assembly of the finger in lower primates. By K. J. VAN ZWIETEN (introduced by J. M. F. LANDSMEER). *Department of Anatomy, University of Limburg, Diepenbeek, Belgium*

In the fingers of many primates, including man, coupling of interphalangeal flexion occurs in different kinds of grips. The structural basis for this coupling is the extensor assembly of the

finger, which, in man and in primates of the Old World, has one medial bundle inserting on the second phalanx, and two lateral bundles alongside the proximal interphalangeal joint which form the terminal tendon for the third phalanx. Proximal interphalangeal flexion displaces the lateral bundles so that they can glide palmarward and relax the terminal tendon. Simultaneous distal interphalangeal flexion thus occurs. Primates of the New World have an extensor assembly without such distinct distribution into bundles as in primates of the Old World. This was demonstrated in Cebidae and Callitrichidae. In these primates it was expected that, in proximal interphalangeal flexion, the lateral parts of their compact extensor assembly would not be displaced palmarward as easily as in the primates of the Old World. Absence of coupled interphalangeal flexion in some kinds of grips would be the result. In their behaviour these arboreal species do show hand postures, in which proximal interphalangeal flexion is coupled notably to distal interphalangeal hyperextension, which leads to effective adhesion of the hands to large branches. Comparable grips occur in *Galago*, a prosimian. Here too, the extensor assembly is compact. This supports the existence of a relationship between a simple structure of the extensor assembly and the prosimian-like nature of interphalangeal coupling.

51. A comparative study of the distal femoral condyle in gorilla and man. By A. J. PALFREY and RUTH St C. GILMORE. *Department of Anatomy, Charing Cross and Westminster Medical School, London and Department of Physiology, Queen's University, Belfast*

Articular surfaces are subject to compressive forces which arise either from the articulating bones or from adjacent tissues. These forces vary with the position of the joint and with the external forces applied to the limb. The shape of the articulating surface is partly determined by these forces and may be described in three ways – by the shape of the border of the articulating cartilage, by the form and by the area of the surface.

The distal femoral condyle has been studied in adult male and female gorilla bones and a comparison made with the human bone. The form of the surface has been assessed by inspection, standardised measurements have been made between defined points on the margin of the surfaces and the area of the surface determined using wax sheets of standard thickness.

The general pattern of the surface and its sub-divisions were similar in the two species so that the majority of the measurements were readily comparable. There is a very pronounced sex difference in the gorilla, the male surface being much larger than the female. The size of the surface in the female gorilla is similar to that of the human bone. In both sexes the medial condyle of the gorilla is larger than that of the human, whereas for the lateral condyle the surface in the female gorilla is rather smaller and in the male substantially larger than the human. In contrast the patellar area is similar in size in the human and the male gorilla, but both shorter and narrower in the female; in both sexes this area is flatter in the gorilla than in man. The area of the surface in the female gorilla (41 cm^2) was within the human range ($43.8 \text{ s.d. } 8.6 \text{ cm}^2$), whereas the area in the male was much larger (74 cm^2).

We are grateful to the Trustees and Curator of the Powell-Cotton Museum, Kent for access to specimens.

DEMONSTRATIONS

D. 1. The enamel of the posterior dentition of East African fossil hominids. By A. D. BEYNON and B. A. WOOD*. *Department of Oral Biology, The University of Newcastle upon Tyne and *Department of Anatomy and Biology as Applied to Medicine, The Middlesex Hospital Medical School, London (Fig. 4)*

In addition to their role in the survival of the individual and in the dietary adaptation of breeding groups, teeth are particularly important to palaeontologists because the structure and properties of enamel makes it particularly resistant to degradation. However, while many studies have dealt with the external and gross morphology of tooth crowns, the internal form of enamel has received little attention.

This demonstration reports the results of a study of the naturally fractured surfaces of the teeth of East African hominids. Forty-seven teeth from Koobi Fora, Kenya, and Olduvai,

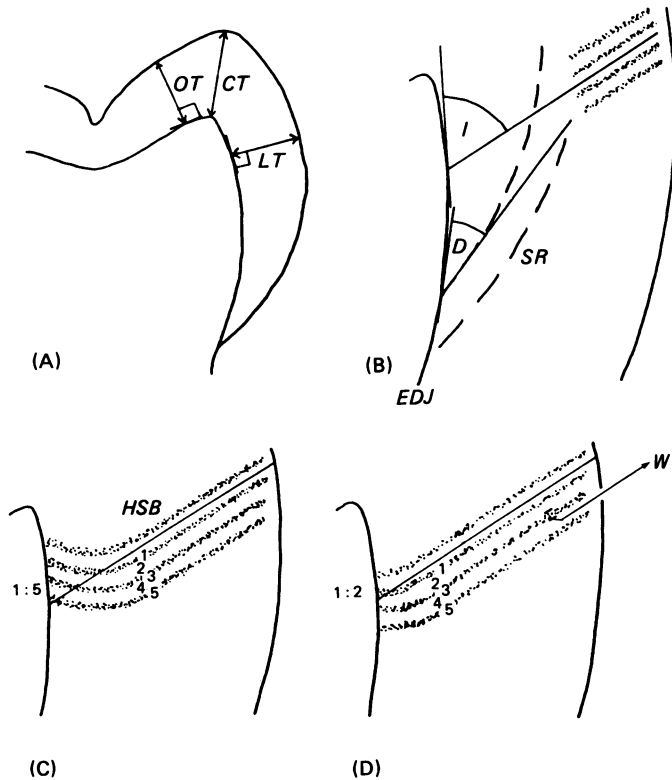


Fig. 4

Tanzania were studied. All but three were permanent, and the 39 molar, six premolar and two fragmented teeth were taken from a total of 31 hominid specimens. The taxonomic category of each specimen was, where possible, established independently by using evidence from the jaws, cranium and the dentition as a whole.

The specimens were orientated with the fracture surface perpendicular to the optic axis of a microscope equipped with a rotatable analyser and either immersed in, or painted with, absolute ethanol. They were illuminated with horizontally polarised incident light, and the analyser adjusted for maximum visualisation of structural features. Enamel thickness was measured on the occlusal (OT), cuspal (CT) and lateral (LT) aspects (Fig. 4A). The average angle of intersection of the Striae of Retzius (D) and the enamel prism direction (I) with the enamel-dentine junction (EDJ), were recorded, together with the degree of curvature and width of the Hunter Schreger bands (HSB) (Fig. 4B, C and D). All measurements are absolute; no adjustments were made for size.

These measurements serve to distinguish between the two largest taxonomic categories in the sample, *Australopithecus boisei* and *Homo* (including *H. habilis*). The teeth of *A. boisei* have thicker enamel (OT 2.8–3.6 mm; CT 2.8–3.9 mm; LT > 2.1 mm), more acutely orientated ($\bar{X} = 52^\circ$) and straighter Hunter Schreger bands, and shallow-sloping incremental lines ($< 30^\circ$). In contrast the teeth allocated to *Homo* tended to have thinner enamel (OT and CT < 2.0 mm; LT < 2.3 mm), more obtuse ($\bar{X} = 62^\circ$) and curved Hunter Schreger bands and steeper incremental lines ($> 30^\circ$).

The slope of the Striae of Retzius and the curvature of the Hunter Schreger bands provide information about respectively the rate of crown coverage by enamel during enamel formation and the degree of decussation of the enamel prisms.

Thus, this study suggests that the enamel of *A. boisei* is thicker, more rapidly accumulated and the prisms less decussated than those of early *Homo*. The presence of prism decussation in primate enamel is believed to be a structural modification to lessen the likelihood of crack

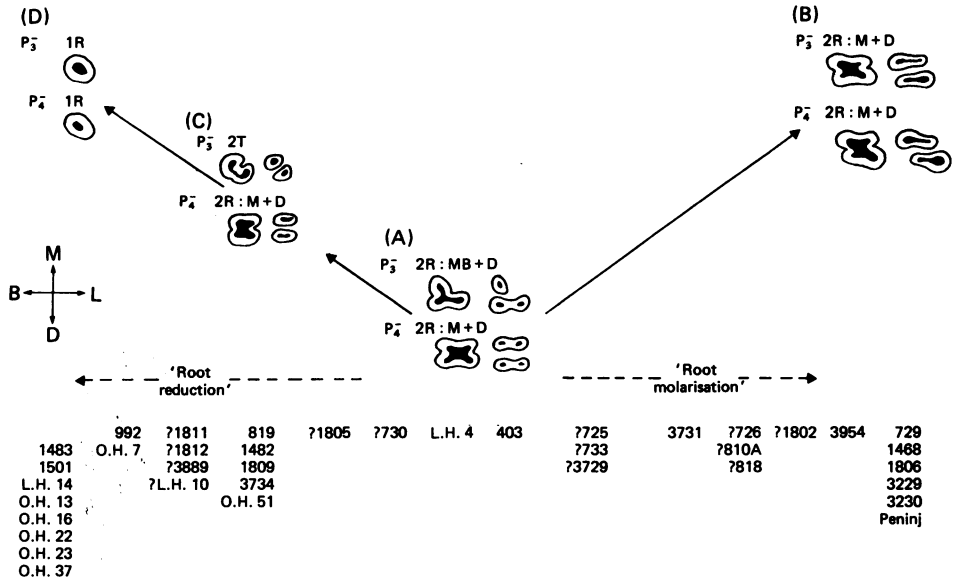


Fig. 5

propagation, and the apparent reduction of such a mechanism in the thick-enamelled 'robust' australopithecines is noteworthy.

We acknowledge the support of The Royal Society, and thank the Trustees of the National Museums of Kenya for permission to examine specimens in their care.

D. 2. Mandibular premolar root form and evolution in the *Hominoidea*. By S. A. ABBOTT and B. A. WOOD. *Department of Anatomy and Biology as Applied to Medicine, The Middlesex Hospital Medical School, London (Fig. 5)*

Among extant and fossil higher primates, high variability in size and shape have been documented for the mandibular premolar crowns, but the root morphology of these teeth has received little attention. This study set out to redress the balance to some extent, by recording the form and variability of the lower premolar roots in extant hominoids and in some East African fossil hominid specimens. In particular, the premolar roots of the fossil hominids were examined for evidence of trends in character states.

Adult lower jaws of *Gorilla* (23 male; 17 female), *Pan troglodytes* (20 male; 11 female), *Pongo pygmaeus* (19 male; 15 female) and *Homo sapiens* (16 male; 18 female) were radiographed (including distortion/magnification correction factor) so that the form and size of the roots could be recorded. The pongid P_4 was always two-rooted, with mesial and distal roots (2R:M+D). The P_3 in *Gorilla* and *Pongo* was two-rooted, with mesio-buccal and distal roots (2R:MB+D); in *Pan* root fusion led to the radiographic appearance of a single root in 29% of specimens. In *Homo sapiens*, all P_4 s were single-rooted (1R), as were the P_3 s except for one individual which showed a Tomes' root form.

The fossil hominid sample comprised 51 specimens from the Plio/Pleistocene sites of Koobi Fora and Peninj in Kenya and Olduvai and Laetoli in Tanzania. The root form of the hominid mandibular premolars could most usefully be recorded using four categories: (A) P_3 2R:MB+D, P_4 2R:M+D; (B) P_3 2R:M+D, P_4 2R:M+D; (C) P_3 2T, P_4 2R:M+D; (D) P_3 1R, P_4 1R. The root forms are shown in cross section in the accompanying Figure (Fig. 5). When more than one root is present the left hand section is the appearance above the bifurcation, and the right hand section below it. Hominid remains are arranged according to premolar form; numbers without a prefix refer to Koobi Fora specimens.

Root form A is typical for extant pongids and, as far as it has been able to be determined, for

Miocene hominoids too (Ward, *Am. J. Phys. Anthropol.* **50**, 1979); it is thus a reasonable hypothesis to suggest that A was the primitive root form for hominids. If so, two trends are detectable in the fossil hominid sample (Fig. 5). One, trend 'root reduction', leads through form C to form D and is a pathway to the root pattern typical for modern man. The second trend, 'root molarisation', leads to form B. The latter pathway is associated with an increase in the size and complexity of the crown, whereas the former is linked to smaller and less complex crowns. Many, but not all, of the specimens along the 'root molarisation' pathway have been attributed to *Australopithecus boisei*, and most of those along the 'root reduction' pathway have been included in *Homo*. However, the apparent variability in the small Laetoli sample, and the position of specimens such as KNM-ER 730 and 1802 are among the intriguing results of this analysis.

We thank the Trustees of the National Museums of Kenya for permission to examine fossils in their care, and we acknowledge the support of the NERC.

D. 3. Functional distinctions between the segments of the proximal tubule of the rat kidney whilst handling a tritiated somatostatin analogue. By M. A. WILLIAMS, J. I. LOWRIE* and J. R. J. BAKER*. *Department of Anatomy and Cell Biology, University of Sheffield and *Ciba Geigy Research, Horsham*

It is well established that the proximal tubule of the rat kidney can be divided into three segments on the basis of tubule cell morphology. Criteria used to distinguish the three segments (S_1 , S_2 , S_3) include phagosome and lysosome number, microvillous length and mitochondrial shape. Recent studies suggest that S_1 is the primary site of glucose and sodium transport, S_2 the major site of protein catabolism and S_3 a site of urea excretion. The three segments have distinct susceptibilities to noxious agents.

We have studied the handling of the somatostatin analogue CGP15425 a peptide of M.W. circa 1100 in the three segments. Under Nembutal anaesthesia one mCi of peptide tritiated on Phe 7 was administered to male Wistar rats 250 g bw generally via the tail vein, as 0.32, 1.6 or 16 mg/kg in 0.9% saline. Five kidneys were perfusion-fixed with 1% glutaraldehyde in Tyrode solution at each of 1, 5, 10, 30, 120 and 240 min. after injection. Kidneys were sliced by and strips taken by an unbiased sampling method. EM autoradiographs were prepared (Ilford L4 emulsion) and exposed for 8 weeks. Autoradiograph images were analysed allowing for crossfire (Williams & Downs 1978), and the results for subcellular compartments scaled to total tissue levels of radioactivity. Tissue contents of the radiochemical were also analysed by HPLC.

Results suggest two types of subcellular pathway. One, through the endosome system to lysosomes appears to represent the fate of large or whole peptides. The other, yielding mitochondrial and cytosol labelling perhaps represents the fate of low molecular weight degradation products. Overall, S_2 segment received the majority of the peptide-derived label. In this segment processing was mainly by the endosome pathway. In contrast, S_3 cells labelled largely via small fragments. S_1 cells showed some labelling via the endosome system, though the lysosomes here never accumulate label to the high levels seen in S_2 lysosomes.

D. 4. Relative importance of viscosity and perfusion pressure during the fixation of proximal tubule cells of the rat kidney for morphometric study. By M. A. WILLIAMS and J. I. LOWRIE, *Department of Anatomy and Cell Biology, University of Sheffield*

It is established that in life, kidney tubules are patent and fluid-filled. To fix them in a realistic conformation, the fixative must be administered whilst the lumina and vascular system are fluid-filled and the kidney turgid (Maunsbach *J. Ultrastruct. Res.* **15**, 166). Several factors must be considered in the design of perfusion fixation protocols. These include fixative tonicity and viscosity and the perfusion pressure. Having previously chosen an appropriate fixative vehicle and tonicity for PT cells (1% glutaraldehyde in Tyrode solution, pH 7.4, 350–370 mosmoles, 37 °C) we report here on the effects of varying fixative viscosity and delivery pressure.

Male Wistar rats 250 g bw under Sagatal anaesthesia plus intramuscular Hypnorm were opened to expose the right kidney for perfusion. Fixative with NaNO_2 added, with or without 2.25% w.v. dextran T40 was applied at 90 or 120 mmHg pressure. Adding dextran yielded viscosity of 2.5 CP, 1 CP without dextran. Six kidneys were perfused via each protocol. They were systematically sliced, further fixed and embedded as small pieces. A stratified sampled

procedure yielding six blocks and six LM micrographs ($S_1 + S_2$ cells) was set up for each kidney. Analysis of variance showed that between animal variance accounted for $> 90\%$ of the total variance of each group. The volume density of lumina and cells in PCT, mean cell volume and certain other structural parameters were estimated. Results were assessed according to a 2×2 factorial design. Mean tubule diameter was very constant, but high perfusion pressure was essential to prevent tubule cell swelling and to minimize between-animal variance for mean cell volume. Addition of dextran appeared to confound the positive effects of high pressure and to offer no advantages.

D. 5. Cholesterol retention in tissue sections: an assessment of different techniques. By M. L. NICHOLSON and W. S. MONKHOUSE. *Department of Human Morphology, University of Nottingham*

The lability of cholesterol during histological processing has long been a problem. Poor tissue retention occurs because of the solubility of cholesterol in dehydrating agents and embedding media. Methacrylate based JB-4 plastic is a water soluble embedding medium and may therefore be of great value in the context of cholesterol retention since the need for complete dehydration is done away with.

In the present study loss of radioactivity was measured when tissues from mice injected with ^3H -cholesterol were processed by different histological methods including processing using JB-4 plastic embedding medium. When a dehydration step is employed before embedding in JB-4 plastic, significant loss of isotope occurs with a final retention of 28% in the adrenal gland and 30% in the liver. Glutaraldehyde fixation followed by Araldite resin embedding is similarly unsuccessful, isotope retention being only 22% in adrenal tissue and 20% in liver. Digitonin precipitation of cholesterol followed by Araldite resin embedding, reportedly successful in the past, yielded poor retention in both adrenal (32%) and hepatic tissues (35%). These methods are therefore unsatisfactory when retention of cholesterol in tissues is necessary.

In contrast, JB-4 plastic embedding without prior dehydration achieved a final retention of 81% in adrenal glands and 63% in liver and thus is a good method for cholesterol retention. It is suggested that this method might be of use in the autoradiographic localisation of cholesterol.

D. 6. Light and electron microscopic observations on rat Leydig cells following a single dose of ethylene-1,2-dimethanesulphonate (EDS). By J. H. SHANKS, J. S. DIXON and R. G. LENDON. *Department of Anatomy, University of Manchester*

Adult male Wistar rats were injected with a single intraperitoneal dose (75 mg/kg) of ethylene-1,2-dimethanesulphonate (EDS) which is known to cause reversible sterility. The animals were killed at selected time intervals after treatment and testicular interstitial tissue of both control and treated rats was examined using light and electron microscopy.

Interstitial tissues of control rats contained perivascular clusters of Leydig cells together with a few macrophages. Leydig cell cytoplasm contained abundant smooth endoplasmic reticulum and numerous mitochondria with tubular cristae, both features being characteristic of steroid-secreting cells.

By $8\frac{1}{2}$ hours after EDS administration a proportion of Leydig cells displayed abnormal lipid droplet accumulation. In addition large membrane-bound fragments of Leydig cell cytoplasm were observed within testicular macrophages. These heterophagosomes were observed in various stages of degradation. Leydig cells observed at 24 hours post-treatment appeared swollen and displayed marked fine structural changes including nuclear pyknosis, gross dilatation of mitochondria and smooth endoplasmic reticulum and cell membrane rupture. By 2 days the degenerative debris had largely disappeared from the interstitium and no recognisable Leydig cells were observed. From 2 to 14 days post-treatment the interstitial tissue was characterised by the presence of cells which closely resembled fibroblasts. No Leydig cells or other cells with typical fine structural features of steroid-secretion were observed throughout this period.

Repopulation of the interstitium by Leydig cells began between 21 and 28 days after EDS administration apparently as a result of differentiation of the fibroblast-like cells. At 21 days numerous cells contained mitochondria with tubular cristae and abundant smooth endoplasmic

reticulum. By 28 days large perivascular clusters of clearly recognisable Leydig cells were observed although even at 35 days many Leydig cells displayed accumulation of lipid droplets, possibly indicative of impaired steroidogenic function.

The results of this study were correlated with previous pharmacological investigations into the effects of EDS on rat testis function.

D. 7. Ultrastructural studies on the differentiation of mouse gonads *in vitro*. By SARAH MACKAY and R. A. SMITH. *Department of Anatomy, University of Glasgow*

The morphological development of mouse gonads *in vitro* has previously only been studied by light microscopy of paraffin sections (Taketo & Koide, *Dev. Biol.* **84**, 1981). We report preliminary results of explant cultures observed by both semithin and ultrathin techniques. The urogenital complex (gonadal primordium and mesonephros) was removed from mouse fetuses aged day 10.5–11 as assessed by developmental criteria.

Cultures were maintained for up to 7 days in Williams E medium supplemented with 15 % fetal calf serum, 2 mM glutamine and with 0.5 % gentamycin present in a 5 % CO₂/95 % air atmosphere at 37 °C. Monitoring by phase contrast microscopy showed explant attachment to the substratum within 24 hours. Further, the continued healthy appearance of explants was checked thereafter on a daily basis until fixation. The use of semithin and ultrathin sections allowed a closer examination and characterisation of structural differentiation, confirming the healthy living appearance with evidence of cell divisions.

Indifferent complexes from several fetuses were cultured together in the same dish in three separate experiments and developed into testes or ovaries. There appeared to be little evidence of inhibition of the differentiation of either sex. In other experiments complexes were also cultured singly. Presumptive male gonadal primordia developed into unequivocal testes with distinct testicular cords and obvious tunica albuginea. Ovarian differentiation was followed to the *in vivo* stage of 'organization' (Upadhyay *et al.* *Ann. Biol. Anim. Bioch. Biophys.* **19**, 1979) showing ovigerous cord development not reported in previous *in vitro* studies.

Future experiments using our relatively simple culture method aim to promote differentiation of primordia obtained from fetuses on day 10. Development of such early primordia has not been successfully achieved to date by others.

D. 8. Optical and electron microscopic characterisation of primary cell cultures isolated from adult mouse dorsal root ganglia. By I. B. MCINNES and R. A. SMITH. *Department of Anatomy, University of Glasgow*

The use of cell models in biomedical research has increased recently, and it is envisaged that this trend will continue. Primary '*in vitro*' systems have many advantages over established cell lines, but often such cultures are difficult to initiate and adequately characterise. This is particularly so for nervous tissue with successful maintenance limited mainly to non-mammalian or embryonic cells. The current project therefore set out to produce cultures isolated from adult mouse sensory ganglia and to determine control requirements as a basis for future studies of neurotoxic agents.

Thoracic and lumbar dorsal root ganglia (DRG) excised from adult male CBA mice were disrupted enzymically overnight with 0.125 % collagenase. This gave better separation than existing methods (Scott, *J. Neurobiol.* **8**, 1977) since it reduced the need for extensive mechanical dissociation. Cultures were prepared in Dulbecco's medium supplemented with 15 % fetal calf serum and 0.5 % gentamycin and maintained in 5 % CO₂/air at 37 °C. Medium replenishment was carried out on alternate days. Cultures were monitored for up to 7 weeks by phase microscopy. Neuron attachment and maintenance was improved by increased glucose concentrations within the range 2–10 g/l.

Twenty four hours following culture initiation isolated neurons and remaining DRG fragments attached to the substratum. Further adhesion and outgrowth was observed during week 1, and with increased culturing non-neuronal cell proliferation effectively produced an extensive meshwork onto which neurons attached and into which neuritic regeneration occurred. If satellite cells were absent or reduced neuronal attachment was less, suggesting a possible role in mechanical support for these cells.

Cell morphology was studied at different ages both in living cultures and also in material fixed for subsequent histological staining and for transmission and scanning electron microscopy. Ultrastructurally; cultured neuron and satellite components compared favourably with cells fixed 'in situ'. Preliminary attempts were made to quantify the neuronal population relating it to total cell number. The potential use in cytotoxicity screening was assessed.

D. 9. Epithelial evagination in the morphogenesis of embryonic chick gizzard. By M. A. QADIR (introduced by P. N. ANDERSON). *Department of Anatomy and Biology as Applied to Medicine, The Middlesex Hospital Medical School, London*

Epithelial evagination has been implicated in the morphogenesis of several glandular epithelial type organs such as the thyroid gland (Hilfer, *Amer. Zool.* 13, 1973). However, little is known about the morphogenesis of the chick gizzard and how it develops into an organ with a large asymmetric lumen from a straight tube. Using semi-thin sections for light microscopy and thin sections for TEM we studied the development of chick gizzard through stages 20-25 (Hamilton & Hamburger, *J. Morphol.* 88, 1951) of embryonic development.

In longitudinal sections, the gizzard at early stage 20 is composed of a straight tube of columnar epithelial cells surrounded by undifferentiated mesenchyme which was condensed on the dorsal side. At stage 21, the epithelial lining on the dorsal aspect evaginates towards the condensed mesenchyme, which was seen to be in close apposition to the bulging epithelium along its entire length.

Examination of various stages of the bulging process by TEM showed that epithelial cells on the distal aspect of the bulge were of elongated columnar type. Apical micro-filament bundles were abundant and seemed to constrict cell apices as cytoplasmic material was seen to 'bleb' towards the lumen. Cytoskeletal elements were oriented longitudinally: microtubules, thick and thin microfilament bundles run along the longitudinal axis of the cells. Most of the nuclei were situated basally. The cytoplasmic organelles were also oriented along the longitudinal axis. Abundant Golgi apparatus, mitochondria, rough endoplasmic reticulum, and ribosomes were found which suggests high metabolic activity. Throughout the stages examined the basement membrane was well developed and composed of a lamina densa, lamina rara interna and lamina rara externa. Mesenchymal cells did not penetrate the basement membrane but remained in very close apposition to it. The cells were connected by gap and tight junctions apically.

The observed cellular arrangement and cytoskeletal orientation within the evaginating epithelium may reflect the underlying mechanisms of the morphogenetic process.

D. 10. Structural and functional changes in innervation of blood vessels in streptozotocin-induced diabetic rats. By T. M. SCOTT and C. R. TRIGGLE. *Faculty of Medicine, Memorial University of Newfoundland, St John's, Newfoundland, Canada*

Associated with the development of hypertension and diabetes in patients and in experimental animals, changes occur in the structure and function of the cardiovascular system. Many authors have suggested that the change which occurs in the relationship between catecholaminergic innervation and vascular smooth muscle is involved in the development of these disease processes. It has been shown that during the development of hypertension in genetically hypertensive rats, vascular smooth muscle sensitivity to noradrenaline and neuronal uptake (Uptake-1) are increased in mesenteric (Mulvaney *et al.*, *Hypertension* 2, 1980) and tail arteries (Laher & Triggle, *Can. J. Phys. Pharm.* 62, 1984), while at the same time the number of nerve profiles innervating the blood vessels was shown to increase (Scott & Pang, *J. Aut. Nerv. Syst.* 8, 1983). Although these studies were not carried out on tissues from the same animals it has been suggested that they may be correlated with the hypertrophy of the arterial media which occurs in hypertension.

In this study we have examined vascular smooth muscle sensitivity, Uptake-1 and pattern of innervation in tissues from the same animals, to determine whether or not changes in these features may be correlated in rats rendered diabetic following treatment with streptozotocin.

Four weeks old rats were injected with streptozotocin (50 mg/kg i.p.). At 4, 8, and 12 weeks after induction of diabetes, the rats were anaesthetised with pentobarbital (30 mg/kg i.p.), and jejunal and tail arteries removed for examination. The jejunal arteries were examined by fluor-

escence microscopy to determine the pattern of innervation. The tail arteries were examined in isolated tissue baths to determine the sensitivity to noradrenaline and Uptake-1 activity.

It was found in these diabetic rats, that there was, in the presence of cocaine, raised post-synaptic sensitivity to noradrenaline in the tissues from rats which had been diabetic for 4, and 8 weeks; that Uptake-1 activity was raised in tissues from the 4 weeks diabetic rats; that 8 but not 12 weeks diabetic rats showed an increase in fibres innervating the jejunal arteries.

It is suggested from this study that changes in vascular smooth muscle sensitivity to noradrenaline, Uptake-1, and pattern of innervation occur during the development of diabetes, but that in considering the involvement of these factors in pathogenesis in the vascular system in diabetes, their temporal relationship might be important.

D. 11. The effects of ionophore A23187 and of perhexiline on various parameters of melanoma cell function *in vitro*. By H. T. SPEAR (introduced by D. R. JOHNSON). *Department of Anatomy, University of Leeds*

α -Melanocyte-stimulating hormone (α MSH), and its second messenger cyclic adenosine monophosphate (cAMP) have well characterised effects upon melanogenesis and proliferation of melanoma cells, increasing the former and decreasing the latter. In addition these substances have been reported to change cell morphology. Studies of non-transformed amphibian melanophores have indicated that the action of α MSH requires calcium ions (Ca^{2+}) at two points: (a) for hormone-receptor binding, and (b) for activation of adenyl cyclase. This latter requirement is fulfilled by extracellular ions which enter the cytoplasm via MSH-activated calcium channels.

A previous study (Sauk, *Pflug. Arch.* **22**, 1976) showed that high concentrations of the ionophore A23187, known to produce an influx of extracellular Ca^{2+} in many cell types, caused B16 melanoma cells in culture to adopt a dendritic morphology within 45 minutes. In the continued presence of the ionophore the cells then assumed a stable rounded or 'epithelioid' morphology. The dendritic processes were reported to be devoid of microtubules. In the present study the effects of A23187 on various parameters of B16 melanoma cells growing *in vitro* have been studied and the findings do not confirm Sauk's observations. No early changes in cell morphology in response to the ionophore were found, but within 24-48 hours the cells adopted a dendritic morphology that remained stable even upon removal of the ionophore. The dendritic processes contained numerous microtubules.

Preliminary studies have also been made into the possibility that, like melanophores, melanoma cells require an influx of extracellular Ca^{2+} to activate adenyl cyclase. Perhexiline, known to act as a calcium channel blocking agent in muscle cells, failed to prevent the changes in melanization and proliferation brought about by MSH. If perhexiline has the same effect on melanoma cells as it does on muscle cells, this finding suggests that the action of MSH on melanoma cells does not require the patency of calcium channels. In addition, concentrations of A23187 known not to transport extracellular ions into cells but to release calcium from intercellular stores, mimicked the action of MSH upon melanoma cells. This evidence suggests that if calcium is required to activate adenyl cyclase in melanoma cells it comes from an intracellular and not an extracellular source.

D. 12. A morphological study of mm. puborectalis. By P. N. JONES (introduced by P. S. WARD). *Department of Anatomy, University of Leeds*

The morphology of the muscular sling around the anorectal junction is being investigated. Dissection suggests that this structure blends with the pelvic floor anteriorly and is separate from it posteriorly. Histologically it has been found to be composed of dense connective tissue at its anterior attachment followed by skeletal muscle which merges into connective tissue and smooth muscle, part or all of which may be considered as an extension of the internal sphincter of the rectum and anal canal.

Two functions are postulated for this muscle complex. Firstly, the acute angle it creates between the lower rectum and anal canal is held to be important in the maintenance of faecal continence (Parks *et al. Proc. Roy. Soc. Med.* **59**, 1966).

Secondly, a sensory role, especially important after removal of visceral afferents in surgical

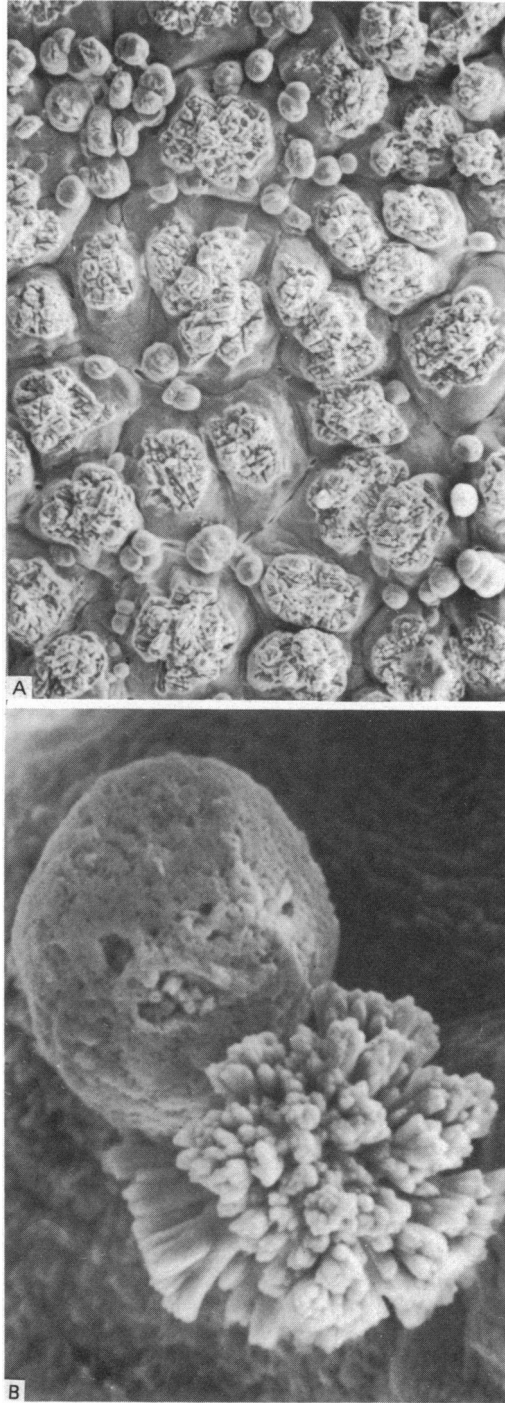


Fig. 6

procedures, has been suggested. Muscle spindles have been considered as the possible sensory receptors (Parks, *Proc. Roy. Soc. Med.* **68**, 1975).

Idiopathic faecal incontinence is a recognised syndrome in the elderly female. In this initial study of females aged 40–70 years at post mortem (with no history of faecal incontinence), particular attention was given to the spindle organs and their innervation. These structures were identified in a few of the muscle specimens only, sometimes unilaterally. Some spindles had a distinctly thickened capsule – an appearance which might be suggestive of reduced functional efficiency.

Current studies are of puborectalis from an age group of females aged 20–40 years, attention being paid not only to muscle spindles but also to other stretch receptors which might function in the reflex control of defaecation.

D. 13. Spina bifida in mediaeval Aberdonians. By GAIL SALUJA, MARGARET BRUCE and JULIET CROSS. *Department of Anatomy, University of Aberdeen*

Spina bifida is a median cleft in the vertebral arch associated with abnormal development of the neural tube. The anomaly may be symptomless but is often accompanied by meningo- or myelomeningo-dysplasias producing disability or death. The precise aetiology of spina bifida is unknown.

This study reports the occurrence of spina bifida in a skeletal series derived from a mediaeval burial ground in Aberdeen. The material comprised the remains of 74 adult individuals with complete or partial vertebral columns, plus a collection of 28 vertebrae which could not be assigned to specific individuals.

A salient feature of the population was the absence of spina bifida from the presacral vertebral column. This contrasts with rates reported for some other populations, e.g. 3.9% of atlases (Allbrook, *Am. J. Phys. Anthropol.* **13**, 1955) and 6% of 5th lumbar vertebrae (Brailsford, *Brit. J. Surg.* **16**, 1929). Lumbosacral spina bifida, involving both the lowest lumbar and the first sacral vertebrae, was not seen in the Aberdeen series, although a rate of 2% has been reported for this anomaly by Hintze (*Arch. Klin. Chir.* **119**, 1922).

Only on the sacrum was spina bifida observed. Complete dorsal non-closure (spina bifida sacralis totalis) was seen in 5% (2/39) of sacra, an incidence similar to that reported by Schmorl and Junghanns (*The Human Spine in Health and Disease*, 2nd ed., Grune & Stratton, New York, 1971). However, partial sacral spina bifida was comparatively infrequent in the Aberdeen group.

These findings are of interest in view of the fact that modern day rates of spina bifida are relatively low in the Aberdeen region (Edwards, *Brit. J. prev. soc. Med.* **12**, 1958). This suggests the prolonged operation of factors associated with low risk for spina bifida. Further investigation may help to identify these factors.

D. 14. The incidence of mammillary layer abnormalities in the eggshells of commercial breeding flocks. By J. WATT and S. E. SOLOMON. *Department of Veterinary Anatomy, University of Glasgow.* (Fig. 6)

In a recent survey of broken eggs from a variety of retail outlets we have shown that 48% of the eggs display inherent structural defects which predispose them to easier breakage during packaging and handling.

The defects are demonstrated using the non-destructive process of plasma etching to remove membranes followed by scanning electron microscopy. Using these techniques six basic types of abnormality have been classified.

- (a) Sulphur and potassium-rich inorganic membrane remnants.
 - (b) 'Sheared' mammillae. During preparation the weak bond between the cap and cone layers becomes evident.
 - (c) Small 'Type A' rudimentary mammillae which are located between normal mammillae and have no apparent association with the membrane fibres (Fig. 6A).
 - (d) Cuffing around the cone layer.
 - (e) 'Type B' mammillae. Occupy a similar area to normal mammillae but do not appear to connect with the membrane fibres.
 - (f) Aragonite. A complete change from the normal crystal modification (calcite) (Fig. 6B).
- From their location and their chemical composition it is possible to implicate specific regions of the oviduct in their initiation.

D. 15. The development of the human midbrain tegmentum with particular reference to the red nucleus. By N. W. MORRELL (introduced by M. N. GHABRIEL), *Department of Anatomy, Charing Cross and Westminster Medical School, London*

Previous work on the early development of the midbrain tegmentum is scarce and the red nucleus in particular has largely been neglected. Although the anlage of the red nucleus has been observed by the end of the seventh week of gestation (Cooper, *Brain* 69, 1946), the site of origin of the rubral neurons remains unclear.

In an attempt to study the patterns of cellular proliferation and migration in the mesencephalic tegmentum serially sectioned human embryos were examined from a closely graded series (fifth to the eighth week of gestation). The mitotic activity of the neuroepithelium at different stages of development was estimated by a surface index method (Smart, *J. Anat.* 111, 1972). During the seventh week of development the neuroepithelium of the midventral region of the basal lamina maintained a relatively high mitotic density while the peak of proliferative activity in the remainder of the mesencephalic vesicle shifted progressively with time dorsolaterally to the alar lamina. The midventral proliferation appears to be an important source of neurons in the midbrain tegmentum.

The results suggest that neurogenesis of the red nucleus occurs during the seventh week of gestation and towards the end of the seventh week the red nucleus becomes apparent. The nucleus arises as a migration of cells from the midventral proliferation and from the lateral angle of the basal lamina. This dual origin may be related to the different adult cell types. Transient and directionally oriented 'fibres' appear in the substratum of the developing mesencephalic mantle layer at the beginning of the seventh week suggesting a role in supporting or guiding migrating neuroblasts.

The subsequent differentiation of the red nucleus involved the appearance of three cell types by the middle of the ninth week of gestation: cells with large, vesicular, pale stained nuclei, cells with small lightly stained nuclei, and cells with small, round, darkly stained nuclei. The red nucleus is also defined by a capsule by the middle of the ninth week. Four main subdivisions of the nucleus, corresponding to the adult arrangement, are apparent by the end of the ninth week.