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Research in the Canine Block

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How we met

Behind the Beaumont Veterinary Clinic, located alongside the Royal Veterinary College, Camden Town, London, is a building called the Canine Block, in which Anne McLaren, Donald Michie and I had adjacent laboratories from 1955-1959. During this time we conducted research on the control of the variation between experimental animals, mouse embryo culture and embryo transplantation, and reproductive aging. Our interactions were one of the most enjoyable experiences of my career. I hope Anne will enjoy my reminiscences about this period.

The events that led to my meeting Anne and Donald began independently, and almost simultaneously, in laboratories in London, UK and Sydney, Australia. I and Peter Claringbold were working in the Department of Veterinary Physiology, University of Sydney on the intravaginal assay of estrogens using mice. At that time the technique of radioimmunological assay had not been developed. The assay we employed was plagued by the large variation in the responses of the individual outbred mice to estrogens. Following the genetic dogma of the time we decided, therefore, to use an inbred strain of mouse in the hope of reducing the variation. Inbred mice, however, were scarce in Australia at that time. We were fortunate to obtain a few breeding pairs of two strains that had earlier been imported from the Institute of Animal Genetics in Edinburgh. After the many months that it took to breed sufficient animals for an assay, we found to our chagrin that these inbred mice were more variable in their response to estrogens than the outbred strain. These results prompted Peter Claringbold and I to write a paper entitled "Why use inbred lines?" which we submitted to Nature. Our paper, which was accepted, also included the observation that the first filial (F_1) hybrids between these inbred lines were more uniform in their responses than the two inbred lines themselves. A few months earlier, unknown to us, a letter, also published in Nature entitled "Are inbred strains suitable for bioassay?" questioned the use of inbred lines to reduce experimental variation. The authors were Anne McLaren and Donald Michie from the Department of Zoology, University College, London. They reported that the duration of narcosis following nembutal injections of inbred lines were more variable than outbred lines and that F_1 hybrids were more uniform than the parental inbred lines.

Soon after I had taken up a fellowship at St. Johns College, Cambridge in July, 1954, I received a letter from Anne and Donald that had been forwarded from Australia. This was the first that I had heard of their work. A meeting between us was soon arranged at Professor Hans Grüneburg's laboratory at University College, London. Little did we know at the time that subsequent events would result in us occupying adjacent laboratories in the Canine Block at the Royal Veterinary College, London. In the spring of 1995 Professor E.C. Amoroso (Amo) persuaded me to accept the position of Senior Lecturer in Physiology at the Royal Veterinary College rather than return to Australia. Part of the conditions of accepting Amo's offer was that I would be given a laboratory in which I could continue my studies on the design of chemically defined media to support the development of embryonic chick bones in organ culture which I had begun with Dr. Honor Fell at the Strangeways Research Laboratory. Amo was able to provide me space on the first floor of the Canine Block. Anne and Donald were supported by the Agricultural Research

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Fig. 1. Anne McLaren at the typewriter in her laboratory in the Canine Block. (1958).

Council in Professor Peter Medawar's Department of Zoology at University College, but were seeking new accomodation for their work. Amo finally offered them a laboratory on the first floor of the Canine Block at the Royal Veterinary College adjacent to mine. Thus, quite inadvertently we became neighbors. During the next four years we extended our work on variation between experimental animals, and came to share other interests, in particular preimplantation development *in vitro* and reproductive aging.

Our laboratories were modest and simply furnished. Anne and Donald had a laboratory, perhaps about 25 x 25 feet with a small office in one corner. One of their doors opened into a corridor on each side of which I had two small laboratories, perhaps 10 x 20 feet each. One laboratory was partitioned into a culture room and a preparation room. The other was a laboratory where I made up media and which also served as an office. I recall that Donald and Anne had one technician. I had a part time technician who was available only when he was not preparing the practical physiology classes, which took most of his time. I have two memories of Anne at that time. On the floor above was a room where the stocks of mice were housed. Anne spent many hours in this mouse room recording and marking newborn mice, and often cleaning their cages and feeding them. I also remember Anne sitting at her typewriter in which a mouse exercised in a wheel fitted into its lid (Fig. 1).

Effect of extreme environments on phenotypic variability

Our collaboration, soon after arriving at the Royal Veterinary College, concerned the effect of extreme environments on phenotypic variability. The variation between organisms produced under different breeding systems has scientific as well as a practical interest. In the same issue of Nature in which Anne and Donald's letter appeared, Grüneberg (1954) published a paper entitled "Variation within inbred strains of mice", which showed that the morphological variation of skeletal elements was greater in inbred lines. Based on the fact that inbred mice can be more variable in their responses than F_1 hybrids from crossing inbred lines, Michie (1955) and McLaren and Michie (1956) hypothesized that increased phenotypic variability could be elicited by subjecting developing organisms to unaccustomed environmental conditions, even if these conditions are uniform. This hypothesis was based by analogy on an earlier generalization of Waddington (1942) that any radical departure from the environment to which a species is adapted impairs morphogenetic regulation and hence causes an increase in phenotypic variability.

Three unused rooms whose temperatures could be controlled belonging to the Department of Hygiene were found on the roof of the College. By getting permission to use these rooms, we could put Donald and Anne's conjecture to the test by studying the postnatal growth of mice born to females that had reared their young in constant but extreme temperatures. It was well known that mice would readily reproduce in the cold environments found in abattoirs (Laurie, 1946). The mice would eat their way into the meat to create chambers in which nests were built from the hessian bags that enclosed the carcasses. Subsequently, others showed that mice would breed at low temperatures provided they were given adequate bedding materials (Barnett, 1954; Barnett and Manly, 1956). It had also been established many years earlier that mice could reproduce at high temperatures (Sumner, 1909).

Permission to use the temperature-controlled rooms was obtained from Professor N.J. Scorgie, Head of the Department of Hygiene. Our experimental strategy was to compare the growth in body weight and tail length of mice whose mothers, 12.5 days pregnant, were placed in three different environments, hot, temperate and cold. They were maintained in their respective environments until four weeks after their litters were born. The hot room was kept at 28°C with trays of water to maintain high humidity. The temperate room was kept at 21°C and the cold room at 5°C. Bedding and material for nest-building was standardized by providing equal amounts of sawdust and cotton wool in all cages. The behavior of the mothers with respect to nest-building was very different in the three environments; those kept in the hot environment pushed the cotton wool out of the cages while those kept in the cold environment made beautiful fluffy nests. These nests provided perfect cover for the mother mice to ambush our fingers when we wanted to inspect the litters. The numbers of young born to each female was recorded, and the newborn mice were weighed the day they were born and at weekly intervals until they were weaned at four weeks old. One of the major effects of the extreme environments was the depression of litter size, which we believe was due to increased prenatal mortality in the females raised in the hot and cold environments. It was well known that there is a highly significant inverse effect between body weight and litter size. Estimates of the linear regression of body weight on litter size in each group were computed and used to adjust the data to a common litter size. After making these adjustments, we found that there were no differences in the rate of growth or development between the mice reared in the hot and temperate environments. The growth of the mice reared in the cold environment, however, was retarded (Biggers *et al.,* 1958b).

The statistical analyses also showed that rearing mice in the extreme environments significantly increased the *between* litter variance of the body weights, particularly in the cold. A test of Donald and Anne's hypothesis, however, necessitated the estimation of the variation in body weight *within* litters at different times after birth. Our statistical expertise was challenged by problems due to the scaling effects arising from the variation in litter size on the body weights of the young. We solved the problem by adjusting the log of the variance of body weights of each litter using estimates of the linear regression of log variance of the body weights on litter size (Biggers, 1958). Two significant findings emerged from these analyses. Within litter variation in body weight was significantly increased by the hot and cold environments, supporting Donald and Anne's hypothesis. As the young grew, however, this variation diminished and became similar to the variation observed in the mice raised in the temperate environment. We suggested that this drop in variation was due to the mice adapting to the extreme environments by calling on physiological compensatory mechanisms (Ashoub *et al.,* 1958).

Anne has a remarkable ability to link old and new findings. This is illustrated in her comments on the recent work of Rutherford and Lindquist (1998) who have studied in Drosophila flies the effect of compromising either genetically or environmentally the heat shock protein Hsp90. They showed that when cryptic genes, normally held in check by Hsp90, are exposed to these experimental perturbations, new variation occurs that can be retained by selection. In an article, entitled "Too late for the midwife toad. Stress, variability and Hsp90," Anne suggests that the effects of inbreeding results in departures from the genetic norm of a species established by evolutionary forces (McLaren, 1999). Further, she suggests that inbreeding creates a stress on the species which compromises the buffering action of molecular chaperones, resulting in the increased phenotypic variability. Crossing two inbred strains could create the necessary uniform heterozygosity which is manifested by hybrid vigor and decreased phenotypic variability.

This period of our work was not without funny incidents. I remember one occasion when Professor Scorgie, who had lent us the temperature controlled rooms, summoned us to his office because of his displeasure about something we had done. The three of us sat in a row facing him across his large desk. He was a man with a short temper and in the course of the interview, after thumping his hands on his desk, he threw up his arms and pitchpolled backwards over his chair. Fortunately we did not have to restrain our laughter for long as the interview came to an immediate close! We also frequently had trouble with Amo procrastinating over giving permission to publish papers. Donald's solution to this on one occasion, when we knew Amo was in the college, was to sit for the entire day on a chair or on the floor at the entrance to Amo's office until the paper we had submitted for his review was approved.

Variance control in the Animal House

In 1955, the complete details of our results used in our earlier independent reports in Nature were published in full (McLaren and Michie, 1955; Claringbold and Biggers, 1955). These findings, showing that F_1 hybrids between inbred lines of mice are phenotypically less variable than the parental strains mice from which they were produced, were confirmed by several other papers that showed the phenomenon was widespread across both animal and plant species. These general results, together with our work described above on the effect of environment on phenotypic variability, led us to write a review published in Nature entitled "Variance control in the animal house" (Biggers, McLaren and Michie, 1958). This review reiterated the earlier recommendations of Michie (1954) and Biggers and Claringbold (1954) that it was unsound practice to use inbred lines in comparative experimental work without good reason. We reached the following general conclusion:

> *"It is useless to ensure a uniform level of some genetic or environmental factor if the level chosen is such as will in itself inflate the responses of the animals themselves..... In general, the conditions which minimize variability seem to be those which promote the general vigor of the colony, whether we are speaking of genetic conditions (for example heterozygosity) or environmental (for example, temperate as opposed to extreme environmental conditions)."*

Our conclusions that inbred animals may be more variable than F_1 hybrids between them and also outbred animals did not go unchallenged. The article in Nature by Biggers and Claringbold (1954) provoked a vigorous response from A.L. Bacharach of the Glaxo Laboratories. In 1923 he imported the first inbred Wistar rats into England primarily for the study of the anti-rachitic substances of cod-liver oil (Bacharach, 1926). He submitted a letter to Nature lambasting our paper which the Editors forwarded to me for comments. My response was equally vigorous and in the end neither was published. In 1960 an article published by C.K. Chai of the Jackson Laboratory, Bar Harbor, Maine, directly challenged our conclusion that the use of F_1 hybrids was preferable to the use of inbred lines in bioassay (Chai, 1960). Chai argued that the slope of the dose-response line (i.e. the linear regression of the response on the concentration of drug or hormone) was the best criterion for selecting the type of mouse used rather than the variation between individual mice. Our response to Chai's argument, also published in Nature, resulted in an acrimonious exchange of letters published at the end of our article (Biggers *et al.,* 1961). Chai's position, however, does point out that factors other than the variation between animals must be considered under some specialized circumstances. Parallel line assays that estimate the potency of hormones or drugs are examples of these cases. The objective of these assays is to obtain estimates of the logarithm of the relative potency. The variance of this parameter is in fact proportional to the ratio of the standard deviation and the slope (Finney, 1964). Nevertheless we maintained that for many experimental investigations the information contained in the inverse of the variance is a primary concern in the selection of optimally bred animals and that there is overwhelming evidence that F_1 hybrids between inbred lines are less variable that the inbred lines themselves and often outbred animals.

Current manuals on laboratory animals do not often refer to our early work on animal variation and its relevance in designing comparative experiments. Our point of view is briefly summarized by Cohen (1979). In contrast, Earl Green, a former Director of the Jackson Laboratory, explicitly states that outbred (random-bred) mice should not be used in experimental work since results of the differences between control and treated groups will inevitably be biased (Green, 1981). He implies that the use of outbred mice cannot avoid these biases and that inbred or F_1 hybrids between inbred lines should always be used. His assertion overlooks the fact that these biases are overcome by using correct randomization procedures.

The best account of the problems involved is given by Festing (1979) who uses the case-study method to illustrate the decisions that have to be made.

Mouse tails

In our work on the growth of newborn mice in different environmental temperatures we collected an extensive amount of data on the elongation of the tails of the young born. The growth in tail length is particularly interesting since the tail functions in body temperature regulation as a heat exchanger. Mice that have had their tails amputated have difficulty in surviving heat-tolerance tests compared to normal mice (Harrison, 1958). The data we collected on tail length remains unanalyzed in notebooks that were stored for many years in Anne's offices in Edinburgh and London. More recently they have been collecting dust in my office at Harvard after she sent them to me on her retirement from her Directorship of the MRC Mammalian Development Unit. Our statistical analyses on the body weight data were done in the Canine Block before the availability of modern digital computers and took many days using analogue desk computers. At the time we were deterred from doing a full analysis of the data on tail length because of the effort involved. Thus the tail length data was only partially analyzed and was never completed because we moved on to other projects.

On perusing our old notebooks while writing this paper, I found the data which are summarized in Fig. 2. It shows scatter plots of the mean tail length against the mean body weight at four weeks of age of each litter of mice born in the three environments. After allowing for the differences in the mean body weights in the litters, it is clear that the tail length is very dependent on the environmental temperature. Many molecular geneticists who routinely obtain mouse blood by clipping off pieces of its tail do not know that rodent tails are complex heat radiators with an extensive complex vascular system that is not uniform throughout its length (Wu et al. 1995). The rodent tail has been extensively used in research on the peripheral vascular system as a model of a linear organ such as the finger. In the rat, which loses 25 percent of its heat through its tail, the perfusion of blood through the tip is ten times greater than in the base, and the perfusion through the middle region of the tail increases eightfold in response to heat stress compared to threefold in the tip and fourfold in the base. I can imagine an investigator, ignorant of thermoregulation in rodents, on observing the death of a transgenic mouse that has lost part of its tail on exposure to heat erroneously concluding that the transgene has a role in the effects of stress.

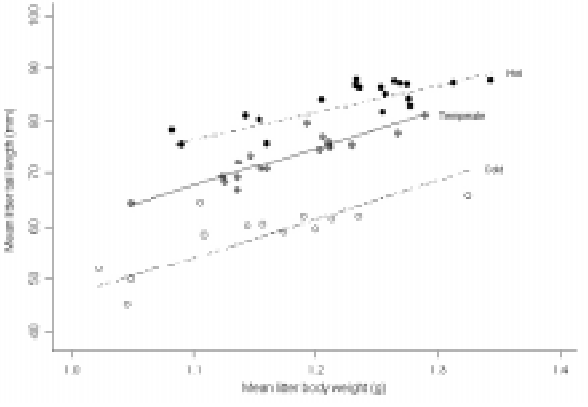

Fig. 2. The regressions of the tail length on body weight of four week old mice raised in cold, temperate and hot environments.

The effect of temperature on the development of the tail presents an interesting developmental problem. I attempted to study the internal morphology of the tails produced in the different environments by preparing longitudinal sections of the tails of the four week old mice. After embedding the tails coiled like a catherine wheel in paraffin, I found it impossible to cut through tough stratified squamous epithelium, dense connective tissue and bone all in one block and obtain unfractured sections. So the work stopped. I would still like to obtain answers to the following questions. Does the regulation of tail length by temperature affect the growth of the tail uniformly along its length or is it confined to the regions where maximum heat loss occurs? Are the changes produced in both the vertebral lengths and the architecture of the vascular beds? What molecular mediators are involved in these processes?

Brave New Mice

The Senior Common Room, located on the second floor of the Royal Veterinary College, was used for morning coffee where informal chats frequently took place. The recently received journals were also on display in this room. I well remember such a morning in 1956 when Anne, Donald and I found, in the latest issue of Nature, a letter by Wesley Whitten, from the National University of Australia, Canberra, reporting the successful culture of eight cell mouse embryos to the blastocyst stage (Whitten, 1956). Although attempts to culture mammalian embryos *in vitro* began in 1913 when it was reported that rabbit blastocysts would expand *in vitro* (Brachet, 1912), little progress had been made in the interim (review: Biggers, 1987). Wes Whitten's letter was a major contribution for it paved the way for the experimental manipulation of the mammalian preimplantation embryo *in vitro*. For his contribution to reach full fruition, however, it was necessary to show that blastocysts produced *in vitro* could give rise to normal mice after being allowed to develop in the uterus of a surrogate mother.

Once again we found an interest in common. A recently published a paper by Anne and Donald described improved methods for transferring mouse preimplantation embryos from one mother to another (McLaren and Michie, 1956). My work was focused on the use of embryonic chick bones as a model for designing chemically defined media for organ culture. All the expertise for extending Whitten's work was available in our adjacent laboratories in the Canine Block. We lost no time in setting up the necessary experiments. After one or two abortive attempts, I succeeded culturing mouse embryos to the blastocyst stage in small test-tubes, repeating Wes Whitten's work. Anne then transferred the cultured blastocysts into uterine foster mothers and we waited to see if young were born. To ensure that our results would be unequivocal, we used genetic markers (eye color) by culturing mice of one strain and transferring them into surrogates of a different strain. Soon after I went for a family vacation to the Isle of White. One day I received a message that a telegram had arrived for me at the local village post office. I remember well the quizzical look on the postmistress's face when she handed me a telegram from Anne which read "Four bottled babies born!" (Fig. 3). In retrospect I am amazed that we succeeded since the environment in the Canine Block was far from ideal. Less than one quarter of a mile away, the frequent coal-burning steam trains leaving St. Pancreas station belched forth their smoke into the atmosphere.

Fig. 3. Anne McLaren and the author examine mice (white) born after being cultured through part of their preimplantation development before being transferred into a surrogate mother.

Our embryo culture and transfer work was published in a letter to Nature on September 27 1958 entitled "Sucessful birth and development of mice cultivated *in vitro* as early embryos" (McLaren and Biggers, 1958). On October 6, our work was reported by Anthony Smith, Scientific Correspondent of the Daily Telegraph under the headline "Brave New Mice" (Fig. 4). Shortly after I received a brief letter from a prominent scientist from Cambridge University reprimanding us for allowing our work to be reported in the popular press. How times have changed! We soon received an invitation from Anthony Michaelis, editor of the popular journal Discovery, to write an article on the implications of being able to culture mouse embryos and successfully transfer them to surrogate mothers (Biggers and McLaren, 1958). The last paragraph of this paper was headed "Brave New World". It read:

> *"It is inevitable that the thoughts of anyone who has worked on the subjects outlined in this article should turn to Aldous Huxley's fantasy "Brave New World", where he describes completely artificial fertilization and development of human embryos. Fortunately we are far removed from this frightening prospect. The study of the cultivation and transfer of embryos is none the less of the greatest interest, both from the point of view of pure science, and because the techniques assocoated with it are potentially of immense value in the investigation of many biological problems in medicine and agriculture."*

How right we were about the implications of our work in science, medicine and agriculture; how wrong we were about the applications in human medicine! The same year our work was published M.C. Chang reported the first account of fertilization *in vitro* in the rabbit (Chang, 1958). Unlike earlier attempts his work was made unequivocal by the use of genetic markers. Thus by this time the stage was set for future work on the combined use of *in vitro* fertilization, embryo culture and embryo transfer. In 1959, Anne moved to the Institute of Animal Genetics, Edinburgh and I moved to the Wistar Institute, Philadelphia.

The first application in mice of the techniques of preimplantation embryo culture and transfer was published by Andrzej Tarkowski, a visiting scientist at the Department of Zoology, University College of North Wales on leave from the University of Warsaw. He described the formation of chimeras by fusing two eight-cell embryos *in vitro* (Tarkowski, 1961). After transfer to surrogate mothers, some offspring developed into hermaphrodites.

In 1962, while Anne was visiting me in Philadelphia, we outlined an agenda for a conference on preimplantation development, and agreed to seek independently financial support. The problem was rapidly solved by Anne who, on her return to London, succeeded in getting the Ciba Foundation to accept our proposal. The conference, called "The Preimplantation Stages of Pregnancy" was held in 1963 at the Ciba Foundation premises on Portland Street in London. I always regret that Wes Whitten was not at the conference due to insufficient funds to bring him from Australia to London. The proceedings were published in a Ciba Foundation volume of the same name as the Conference (Wolstenhome and O'Connor, 1965). Because of the policy of the Ciba Foundation to publish all conferences under the name of the Director of the Foundation and a professional editor, Anne and I were not editors. The conference was a great success and the book has had a significant influence stimulating the study of the biology of preimplantation development and its applications. In 1996 the book was recognized as having had special significance by the Times (of London) Higher Education Supplement (March 8, 1996) in its series "Speaking Volumes", in which Anne summarized the origin and content of the Conference.

Mouse strains and embryo culture

Following the Ciba Foundation Conference, the two cell block was recognized. Mice zygotes from outbred and inbred strains would develop into the two cell stage and arrest, while late two cell embryos would continue to develop *in vitro* into blastocysts. The arrested two cell embryos could be rescued by transfer into organ cultures of the oviduct (see Biggers, 1998, for a review). The use of F_1 hybrid mice became important when Whitten and Biggers (1968) showed that the two cell block could be overcome by fertilizing their ova. The effect is undoubtably due to heterosis, and F_1 hybrid ova are now frequently used in studies of *in vitro* fertilization and embryo culture. Often sperm from F_1 hybrid males are used because of their vigor. It should be

Science and the Citizen

Brave New Mice

Our Science Correspondent writes about test-tube 'babies,' an important eclipse, and H(for hurricane)-proof houses.

By ANTHONY SMITH

WITH the help of authors who have delved into the future, and with the aid of the bolder sort of newspaper headline, the term "test tube baby" has passed into the language.

So deeply has it become lodged that there is a tendency to think mammalian embryos have actually been raised in some kind of container other than the natural one.

It is known that spermatozoa can be successfully kept in a tube, and from this has come the belief that scientists have gone a step further. A very recent experiment carried out at the Royal Veterinary College, London, shows that scientists have indeed taken a pace forward and that the belief is one stage better confirmation.

For two days mouse embryos were kept and cultivated on a synthetic medium before being returned to suitably receptive female mice. By present standards such a long time means a considerable advance in technique. If methods can be improved still further, this work will be a wonderful way of detecting the influence of the uterus on the developing embryo, and will also enable scientists to experiment on the embryo in a way that is impossible when it is within its mother.

The London mice, which spent so long outside natural care, were looked after instead by Dr. J. D. Biggers and Dr. Anne McLaren. They were obtained, when only 8-16 cells large, from the oviducts of mice which had been mated 2½ days previously. These donating mothers had to be killed, and their immature embryos were then cultured at 37deg. C. in a solution of glucose and albumen.

After 18 hours 87 per cent. of the mouse embryos had developed into blastocysts, the ship-shaped conformation of cells which is a stage nearly all multicelled animals have to pass through extremely early.

These blastocysts were then transferred to females which had been mated 2½ days before. As the embryos which had spent time outside suitured artificially were all due to become albinos, there was no question of muddling them after they developed. With the developing mice already resident in the foster-batch of mothers. One-fifth of all the albino embryos continued to develop in their new mothers, a ratio that is about normal, for a high mortality of blastocysts exists even with developing embryos untouched by the hand of scientists.

Most of these brave new world mice were killed at birth or shortly before to investigate their characteristics. But two are still alive. A few weeks old, they appear, according to the experimenters, "in no way affected by their unusual history."

Fig. 4. Report in the London Daily Telegraph (Oct. 6, 1958) of the birth of mice that had spent part of their life developing in a test tube.

noted that when F_1 hybrid ova are fertilized with F_1 hybrid sperm of the same strain, the advantages of the F_1 zygote only lasts until the paternal genes have been activated. After this time the embryos become the second filial (F_2) generation. Nevertheless, the use of ova from expensive inbred lines is often not necessary. Much of the work on the development of chemically defined media for the culture of mouse embryos has been successful using ova from outbred mice.

Factors affecting phenotypic variability are of interest because of the development of cloning techniques. Frequently it is claimed that genetically identical clones could be exploited in the design of experiments. I am unaware of any studies on the phenotypic variation in offspring produced from preimplantation embryos manipulated *in vitro,* other than that reported by Papaioannou et al. (1989). The variances of the body weight and tail length of the young that developed from genetically identical half embryos produced by destroying one blastomere at the two-cell stage were significantly greater than those from the control animals. There has been much ado about the recent production of a cloned rhesus monkey from one blastomere from a four-cell stage (Chan *et al.,* 2000). To produce genetical identical quadruplets by splitting a four cell stage in this species requires that each be transferred to a separate surrogate mother. What effect four different uterine environments may have on the phenotypic variability between the quadruplets is unknown. The factors that could influence the phenotypic variability in these types of manipulations and also the statistical limitations involved in the use of identical twins in the design of comparative experiments has been discussed by Biggers (1986) and Papaioannou et al. (1989).

John Hunter's unilaterally ovariectomized pig

One lunch time at the Royal Veterinary College, probably in 1957, the question was raised as to whether the total number of young a female could produce was limited by the number of oocytes that could be recruited from the total oocyte store. The subject had come up at a conference that Donald and Anne had attended. The question had been addressed long ago by the eminent eighteenth century anatomist John Hunter (Hunter, 1787). He observed two sows, one unilaterally ovariectomized and the other left intact as a control. At the end of their reproductive lives, that is when they stopped breeding, he observed that the intact sow had produced approximately double the number of piglets as the unilaterally ovariectomized sow. The unilaterally ovariectomized sow bred for six years producing 76 young and the intact sow for eight years producing 162 young. John Hunter wrote in his paper, with an added footnote:

> *"If the observations should be considered as depending on a single experiment, from which alone it is not justified to draw conclusions, I have only to add, that the difference in the number of pigs produced by each was greater than be justly imputed to accident, and is a circumstance certainly in favour of the universality of the principle I wished to ascertain."*[1]

By the end of lunch Anne and I decided to put the question to a critical test using mice.

Forty seven outbred female mice were randomly divided into three groups. Twenty three mice were left intact, and two groups of 12 mice were unilaterally ovariectomized on the left and right sides, respectively. After one week each female was placed with a male. All females were then kept as monogamous pairs until they ceased to breed. Thus the females were reproducing under maximum pressure since they almost always became pregnant again within 24 hours at each postpartum estrus. The experiment lasted longer than we had anticipated and Anne and I had left the Royal Veterinary College for our new appointments before the work ended. We persuaded Colin Finn, a faculty member of the College to complete the work. Our findings were eventually published (Biggers *et al.,* 1962a,b).

[1] It may be thought by some that I should have repeated this experiment; but an annual expense of twenty pounds for ten years, and the necessary attention to make the experiment complete, will be a sufficient reason for my not having done it."

The intact mice bred for 435 days and produced a mean total of 115 young in a mean of 16 litters. In contrast, the ovariectomized mice bred for 332 days and produced a mean total of 64 young in a mean of 11 litters. The ratio of total young produced by the ovariectomized females was 0.56 of the total young produced by the intact females, which is not significantly different from a theoretical value of 0.5. John Hunter obtained the same result using two pigs about 175 years ago!

To understand the effect of aging on the production of young in a polytokous species requires the determination of the relation between the number of young in each litter and the litter order. We called this relationship the curve of reproductive performance. The curve is made up of three segments, an initial segment in which the litter size increases, a middle segment in which the litter size stays constant, and a final segment in which the litter size falls. The reproductive performance of each female is summarized by one of these curves with a specific number of litters. We eventually computed a standardized curve of reproductive performance, a statistical challenge which was solved with the advice of Donald Michie. The shapes of the standardized curves of reproductive performance were similar for the intact and unilaterally ovariectomized groups. Initially the litter size was the same (6.5 young). Thereafter the litter size in the intact females rose to a plateau of 9.5 young which lasted until the 7-8th litter, after which a decline in litter size occurred. The plateau was lower in the unilaterally ovariectomized females and lasted until the 5-6th litter after which a decline set in. The rate of decline was the same in both the intact and unilaterally ovariectomized females and occurred at a rate of approximately one young per litter order. We concluded that the earlier onset of the decline in the unilaterally ovariectomized mice is due to overcrowding in the uterine horn. More ova are shed by the single ovary and the embryos produced are confined to the ipsilateral uterine horn since the lumens of the two uterine horns in mice are not connected. As a result this uterine horn undergoes premature functional aging. We also concluded that the similar rates of decline in litter size in both groups is not due to a lack of ova but due to factors that result in embryonic loss. Thus, in the mouse whose females are reproducing at maximum capacity, the uterus eventually becomes the limiting factor in the production of young.

Our results also shed information on the control of the duration of gestation in the mouse (Biggers *et al.,* 1963). It is well known that the duration of gestation is inversely related to the size of the litter. Our results showed that this relationship was not affected by unilateral ovariectomy which causes overcrowding in the functional uterine horn. Thus the length of gestation in the mouse is controlled by the total number of fetuses carried by a female rather than their density in a restricted region of the genital tract.

Scientific environment

Our work in the Canine Block was done in a long departed scientific environment. We did not have to seek research grants and worry about the sometimes capricious decisions of peer review. Anne and Donald's work was supported by the Agricultural Research Council and I do not believe they were restricted in the research they could do. I was financed by money provided from Department of Physiology funds by Amo who was more than generous with his support. To experiment with animals we had to be individually licensed by the Home Office but these licences were general and not specifically tied to research projects as now occurs in the USA. There was no institutional committee to satisfy about each research project. As a result we were able to initiate the three research areas in which we worked with little delay.

It was common practice to list authors of publications in alphabetical order. On looking at the bibliography to this paper, I am embarrassed by seeing the numbers of our papers in which I was the first author. We were all equal contributors. Some journals such as the Proceedings of the Royal Society insisted on this practice. We encountered this rule when we submitted one of our papers on the effects of environment on phenotypic variability to the Editor of the Proceedings of the Royal Society (Ashoub *et al.,* 1958). Ashoub was an Egyption Fellow in the Department of Hygiene who assisted us in our work. On the outbreak of hostilities between Britain, France and Israel with Eygpt over control of the Suez Canal, Asboub was recalled to Eygpt and we lost contact with him. Our request to the editor that the address of the first author should be one of us in Britain was firmly declined. We now live in an age where young people fight for first authorship, fueled by search committees for jobs making paper counts including first authorship. Publish or perish did not exist in those days. But in an age where more and more research is done by interdisciplinary groups first authorship again has little significance.

During the course of the work on the environment and phenotypic variability we were informed that our research might impinge on the independent work a graduate student was doing at another institution. This could have serious consequences for the student since the rigid requirements for awarding the degree at British Universities is that the work be original. A request was made that we delay the full publication of our work until the student had completed his thesis. This we did, an action that I cannot imagine would be done today.

What a wonderful time this was in my career being closely associated with two people with whom I could collaborate and share scientific interests. Our approaches to research were particularly compatible because we had all been exposed to the Fisherian biometrical way of experimentation. Anne, and Donald in particular, came under Fisher's influence through genetics and I through the field of bioassay of hormones and experimental design. How fortuitous our laboratories were adjacent. I hope Anne also remembers with great pleasure our association in the Canine Block.

Summary

This article, in honor of Dr. Anne McLaren, describes research done during 1955-1959 in the Canine Block at the Royal Veterinary College, London. During that period, Anne in collaboration with the author demonstrated that cultured mouse preimplantation embryos could develop into normal mice after transfer to surrogate mothers. We also studied in depth the control of variability of experimental animals and reproductive aging. In recalling this period, I reminisce about Anne and the scientific environment in which the research was done.

KEY WORDS: *Anne McLaren, phenotypic variability, preimplantation embryo culture, reproductive aging.*

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