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Findings and Conclusions: Induced parthenogenetic development is very successful in lower animals such as invertebrates, but in vertebrates, parthenogenones usually do not survive until hatching, or in mammals, until birth. We are barely on the threshold of knowledge in this field, and when there is a break it is possible that many desirable characteristics of animals that are sometimes lost through sexual reproduction, can be retained and even produced in large numbers if desired.

ADVISER'S APPROVAL L. Herbert Bruneau

EXPERIMENTAL METHODS FOR INDUCING PARTHENOGENESIS

By

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Bachelor of Science

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Stillwater, Oklahoma

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Submitted to the faculty of the Graduate College
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in partial fulfillment of the requirements
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ADVISER'S APPROVAL L. Herbert Bunyan

EXPERIMENTAL METHODS FOR INDUCING

PARTHENOGENESIS

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CHAPTER I

INTRODUCTION

Parthenogenesis is derived from the Greek words meaning "virgin birth." This is a specialized type of asexual reproduction as compared with sexual reproduction.[1] Throughout the plant and animal kingdoms, sexual reproduction is the most common, and the generally accepted, method of producing offspring. Sexual reproduction occurs when an egg, which is sometimes called the ovum or female gamete, is stimulated to develop into a new organism after fertilization by a sperm, which is sometimes called a spermatozoan or male gamete. The fertilization process is described as being the fusion of the egg and sperm.

The phenomenon of parthenogenesis, or virgin birth, in the vast majority of cases involves the egg, but not the sperm. An embryo develops from an egg without fertilization, even though the embryo may, or may not, eventually develop into an adult. This developing parthenogenetic organism is called a parthenogenone. The general rule is female parthenogenesis, the developing gamete being the egg, though male parthenogenesis does exist in certain groups of lower plants. In certain species of algae, in which the male gamete closely resembles the female, offspring develop without the female. Examples of parthenogenetic development have been

[1]"Parthenogenesis," Chambers Encyclopedia, 10 (1967), p. 480.

found in most groups of plants and in many groups of animals. This development of the embryo from an unfertilized egg occurs naturally or spontaneously; or it may be induced artifically or experimentally by man.

Natural parthenogenesis is the spontaneous development of the unfertilized egg into an embryo and not having been stimulated by man. This is the exclusive method of reproduction in some species of plant lice, parasitic wasps, and thrips in which males are unknown. It is thought to be an occasional method of reproduction in some members of all the major animal groups, except the vertebrates and echinoderms. In still other groups of aphids, rotifers, water fleas, and many more animals, parthenogenesis alternates with sexual reproduction. In some members of the insect orders, hymenoptera, hemiptera, or thysanoptera, any egg is capable of developing parthenogenetically, or after fertilization, the fertilized eggs are capable of producing females, the unfertilized eggs, males.

There are many types of natural parthenogenesis described.[2] Cyclic parthenogenesis is the name applied to the groups of aphids, rotifers, and water fleas, described in the preceding paragraph in which sexual and parthenogenetic reproduction alternate. In rotifers any successive generation may include both parthenogenetic and sexual females. The parthenogenetic female produces large eggs, which remain unfertilized and develop into either parthenogenetic or sexual females. The sexual female produces small eggs, which may develop without fertilization and yield females. In aphids several parthenogenetic generations follow the fertilized egg, which hatches in the Spring; during the Spring and

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[2]"Parthenogenesis," The Encyclopedia Britannica, 17 (1967), p. 409.

Summer, wingless or winged females, or both, are produced parthenogenetically. As Autumn approaches, sexual forms of both male and female appear and mating takes place. The males, being born mostly of wingless parthenogenetic females, and the females, being born from winged parthenogenetic females. The eggs from these matings hatch the next Spring, and the cycle repeats. The water flea cycle is more flexible and may produce either males or females by parthenogenesis, or by eggs, depending upon the environment.

In rudimentary parthenogenesis,[3] which is found in most animal groups, the unfertilized egg undergoes only slight development, because chromosome regulation is lacking. A more advanced type is called facultative parthenogenesis. This type, which is common among fruit flies and the stick insects, gives rise to parthenogones, but normally these animals reproduce sexually. In geographical parthenogenesis, shown among the woodlice and other insects, the parthenogenetic form occupies a different region from that populated by its ancestral sexual form and is sometimes polyploid. That is, its chromosome number being a multiple of the ancestral number. Another condition that is somewhat between sexual and parthenogenetic reproduction is called pseudogamy. This is exhibited in some nematodes and insects where the spermatozoan merely activates the egg into successful development, but has no effect on the inherited characteristics.

Remarkable cases of experimental parthenogenesis have been induced by physical and chemical techniques. This was initially discovered in 1886 by A. Tichomiroff, working with heated eggs of the silk moth. In

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[3]"Parthenogenesis," Chambers Encyclopedia, 10 (1967), p. 481.

the early 1900's, T. H. Morgan and A. D. Mead showed that development could be initiated in unfertilized eggs of certain sea animals.[4] Later, extensive investigations by many workers showed that in practically all the main groups of animals, normal development could be obtained by artificial activation of the unfertilized egg, although the methods of treatment may vary considerably from one species to another. This treatment might include pricking eggs with a needle, temperature shock, centrifugation, hypertonic or hypotonic solutions, placing eggs in vitro, subjecting them to colchicine, radiation, or even injections of anesthetics.

The purpose of this paper is to bring together information available on methods of experimentally inducing parthenogenetic reproduction.

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[4]"Parthenogenesis," The Encyclopedia Britannica, 17 (1967), p. 410.

CHAPTER II

MECHANICS OF PARTHENOGENETIC DEVELOPMENT

Normally an individual receives one set of chromosomes from the female parent and one set from the male parent. In parthenogenesis all of the chromosomes, and genes, come from the female parent. The offspring may have one set of chromosomes, the haploid number, or two sets, the diploid number; or the sets of chromosomes may be duplicated many times, which results in polyploidy.

Important differences in parthenogenesis are related to the number of chromosomes in the developing egg. In most higher animals, whose body cells contain what is known as the diploid number, or normal number of chromosomes, such as man's forty-six chromosomes or twenty-three pair, the reproductive cells go through two maturation divisions. During one of these divisions the like, or homologous chromosomes, after pairing, separate without division. Because of this reduction division, the mature germ cells have half the normal number of chromosomes. This is called the haploid number. At times these haploid eggs may develop parthenogenetically. More commonly, however, parthenogenetic eggs omit the reducing one of the two maturation divisions, and therefore are diploid.

In animals, even after reduction division, the normal, or diploid, number of chromosomes may be restored in parthenogenesis by duplicating the chromosomes at the end of reduction division. The diploid number

may also be restored by fusion of the egg nucleus with the nucleus of the second polar body, which is the very small one of the two cells, produced by the second division. Also fusion of the two cleavage cells could occur.

In the case of experimentally induced parthenogenesis, the treatment usually blocks one of the cell divisions after the chromosomes are already duplicated. Since they duplicate again before the next division, the embryos have two sets of chromosomes, even though the two sets are identical. This fact explains the lack of vigor typical of these experimentally produced parthenogenetic embryos. Some of the genes of diploid species are usually detrimental or lethal when homozygous, or present in two doses. In cross-fertilization the set of genes from the male parent usually compensates for any defective genes inherited from the female. In groups normally reproducing by parthenogenesis, such genes have largely been eliminated from the population.

Sex is determined by the inheritance of a special chromosome (X-chromosome) from the female and a Y-chromosome from the male parent. Sperms may contain either an X-chromosome or a Y-chromosome, but all eggs contain an X-chromosome. The union with an X-carrying sperm produces a female embryo and with a Y-carrying sperm, a male embryo. In parthenogenesis the embryos would get an X-chromosome with each set of chromosomes and all would be expected to be female.

In organisms that reproduce by parthenogenesis, evolution is restricted because, in the absence of chromosome pairing, recombination of characters does not occur. Ineffectiveness of selection within parthenogenetic lines of descent indicates that changes are rare, though if crossing over (the exchange of fragments between homologous chromosomes)

ever occurs, recombination of characters is still possible, permitting a slow evolution. In species reproducing alternately by the parthenogenetic and sexual methods, parthenogenesis may be an evolutionary asset. When recombination in the sexual period produces an individual that fits some special niche in the environment, that individual need not stand alone in holding its place. By parthenogenesis it may produce hundreds of individuals having the same genetic constitution, many of which, even after high accidental losses, may well survive.

CHAPTER III

METHODS OF PHYSICAL ACTIVATION

The cold treatment, or chilling, of unfertilized eggs has been experimented with in many of the major divisions of animals. This treatment includes chilling the eggs outside the animals' bodies and inside the Fallopian tubes of mammals. It also includes allowing the development of the egg to take place in vitro (in a glass container, such as a test tube), or in situ (in its natural position.)

In one such experiment fish eggs were subjected to a cold shock, and foreign spermatozoa was introduce. Three embryos developed and were found to have the haploid number of chromosomes. Thus is was assumed that the cold treatment or foreign spermatozoa, or both, activated the egg.[1]

In amphibia the cold shock treatment is the standard method of inducing haploid development. M. Fischberg activated newt eggs by this method in 1947. The method has also been adapted to mice. In one experiment ice was placed directly on the Fallopian tubes of female mice. After three and one-half days, three embryo, out of a possible eighty-five, were recovered. The low incidence of development probably meant that this treatment is not very effective in mice.[2]

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[1] R.A. Beatty, Parthenogenesis and Polyploidy (Cambridge, 1957), p. 25.

[2] Ibid., p. 57.

Chilling of rabbit eggs seems to be one of the major experimental projects in parthenogenesis. These experiments should result in valuable information for future use, since the rabbit is one of the few mammals in which parthenogenetic development has been induced. In one such experiment, artificially ovulated eggs were cooled shortly after ovulation within the rabbit's own Fallopian tubes. This was accomplished by exposing the tubes and enclosing their mass in a special cooling apparatus. This experiment yielded some true parthenogenetic embryos, all females. But from this cooling experiment, there came only one embryo that developed to term, out of about twenty-five hundred rabbits subjected to the treatment.[3]

Cooling was carried out in a completely different manner by Marie Chalmel. Fertility of the rabbit oocyte after activation by cold was studied, and the reaction of the parthenogenetic egg was compared with that of the normally fertilized egg. In this experiment 430 rabbits from non-selected breeders were used. Cooling the oocyte of the rabbit in vitro to 0 degrees C. for one minute resulted in partial activation and the formation of a diploid nucleus. The percentage of oocytes, activated and fertilized, was twenty-nine per one hundred in vitro.[4]

Heat treatments are, so far, less successful than chilling for parthenogenetic development. Haploid parthenogenesis has been reported in unfertilized eggs of the silkworm and also one of the annelids. It is also reported that amphibian eggs have responded to heat treatments. M. C. Chang heated unfertilized rabbit eggs in vitro at 38 degrees C.,

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[3]Gregory Pincus, "Fertilization in Mammals," Scientific American, 184 (1951), p. 47.

[4]Marie Clair Chalmel, "Possibility of Fertilization of Parthenogenetically Activated Rabbit Eggs," Biological Abstracts, 46 (1965), p. 3150.

and then transferred them into host females at 24 degrees C. It was found that fifteen percent cleaved into two to seven cells, but no further.[5]

Even though the heat treatment is generally unsuccessful in higher animals, sea urchins and starfish eggs can be made to develop by heating them to certain temperatures.[6]

Centrifugation is sometimes thought of as being a method, but from information gathered, it appears that this physical process requires a spermatozoan nucleus. These are fused together and sometimes develop into triploids.[7]

Another physical method that induces asexual parthenogenetic development is activation of frog eggs by pricking them with a needle. This was a method used in which 202 out of 413 eggs (49%) cleaved without delay. There was an experiment with a human ovum obtained from the Fallopian tube fifteen days from the onset of menstruation. It was cultured in human blood serum with a little ethyl ester of acetic acid. After pricking the egg, it began cleavage, but then it became degenerate and unsuccessful in its attempt to develop.[8]

Strictly speaking, the next subject is not termed development by parthenogenesis, but by a special variation of it called "gynogenesis"

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[5]R. A. Beatty, Parthenogenesis and Polyploidy (Cambridge, 1957), p. 27.

[6]A. Monroy, "Fertilization of the Egg," Scientific America, 183 (1950), p. 48.

[7]R. A. Beatty, Parthenogenesis and Polyploidy (Cambridge, 1957), p. 58.

[8]A. Monroy, "Fertilization of the Egg," Scientific American, 183 (1950), p. 47.

or "pseudogamy". Here the spermatozoan enters and activates the egg, but the sperm nucleus takes little, or no, part in the development. The sperm is genetically inactivated by agents, such as X-rays, radium irradiation, ultra-violet rays, dyes, and nitrogen mustard gas. Even though the spermatozoan is genetically inactivated when the spermatozoan enters the egg, we also must consider the sperm's tail, centriole, extra-chromosomal chromatin, and also cytoplasm which may contain self-perpetuating bodies. Any of these might effect egg development. Also the inactivated nucleus may provide disorganized chromatin capable of being used by the egg.[9]

Experiments in pseudogamy have been carried out with the eggs and sperm of the leopard frog, after treatment with X-rays, and embryos have developed. In yet another project, there were obtained newt larvae with the haploid number of chromosomes after ultra-violet radiation of the spermatozoan. In mammals, an experiment with sheep was carried out. The spermatozoa were suspended in a medium containing trypaflavine, a chemical dye. These spermatozoa were then inseminated into females. Only early activation was observed in these ovum from sheep.[10] Investigations with mice treated with nitrogen mustard resulted in divisions of a few egg cells up to thirteen total cells with the haploid number.[11]

From all reports of parthenogenesis that result from physical activation, it thought that these methods will usually achieve a partial

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[9]R. A. Beatty, Parthenogenesis and Polyploidy (Cambridge, 1957), p. 18.

[10]Ibid., p 32.

[11]Ibid., p 33.

development of the egg, and only in a very few isolated cases of the hundreds of thousands of eggs activated, have resulted in a fully developed embryo. Success is usually achieved in lower groups of animals, such as invertebrates, but the rate of cleavage invertebrates is extremely slow and very short in duration.

CHAPTER IV

METHODS OF CHEMICAL ACTIVATION

It has been discovered that various chemicals will cause eggs to develop parthenogenetically. The most well known is the fowl pox vaccine, used primarily in chickens and turkeys. The photograph of a parthenogenetic turkey has circulated world-wide, and the turkey is believed to be the oldest surviving parthenogenetic poult. At the time its picture was taken, it weighed twelve pounds and was 161 days old.[1]

It is not clear if the activating agent is the vaccine itself, or a contaminant that it may contain. Whichever it is, has caused an increase in the number of eggs showing spontaneous embryos.

Studies were initiated at the Agricultural Research Center, Beltsville, Maryland, in 1953 to determine which factor, or factors, were responsible for the parthenogenetic development encountered in the eggs of chickens and turkeys. Since these studies were initiated, more than thirteen thousand infertile chicken eggs have been incubated and examined for parthenogenesis. These eggs were produced by chickens of four different breeds: New Hampshires, Barred Rocks, Rhode Island Reds, and Dark Cornish, as well as two crosses involving New Hampshires and Dark Cornish. Data obtained showed an inherited tendency on the part of certain individuals, as well as on the part of different breeds, to

[1] "Vaccination Increases Parthenogenetic Chances," Science Newsletter, 72 (1957), p. 291.

produce eggs that will develop parthenogenetically. The eggs of Dark Cornish and Cornish crosses were the only ones encountered showing any appreciable amount of parthenogenesis that could be detected macroscopically. The development in the case of chicken eggs consisted typically of a limited growth of membranes. These data also indicate that this tendency can be increased or decreased by selective breeding. It was found that a virus may be involved in the initiation of parthenogenetic development in the eggs of Dark Cornish chickens.

The eggs of thirty-five Dark Cornish females were examined before and following vaccination with the fowl pox virus. The number of eggs showing parthenogenetic development was fifteen times greater after vaccination. The highest incidence of parthenogenesis was encountered in eggs laid thirty to sixty days after the birds had been vaccinated.[2]

A further indication that fowl pox vaccine may be involved in the initiation of parthenogenesis is furnished by data obtained with turkey eggs. In these tests a total of 3110 eggs laid by two groups of turkeys were examined for parthenogenesis.

One group was composed of sixteen non-vaccinated birds, the other forty-nine turkeys that had been vaccinated for fowl pox.

A total of 738 eggs was produced by the sixteen non-vaccinated turkeys. Of these, 180 (24.4%) showed parthenogenetic development.

The forty-nine vaccinated females produced a total of 2362 eggs during the same period. Parthenogenetic development occurred in 750 (31.8%) of these eggs.

The records of these two groups reveal that a significantly higher

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[2]M. W. Olsen, "Fowl Pox Vaccine Associated with Parthenogenesis in Chicken and Turkey Eggs," Science, 124 (1956), p. 1078.

percentage of parthenogenesis occurred in the eggs laid by the vaccinated group of turkeys, 31.8 percent, as compared with 24.4 percent of total eggs tested.

It is not known, at present, whether the fowl pox virus, as such, is the sole agent initiating parthenogenesis, or whether some contaminant which may be present in the vaccine is also involved. Neither is it known just how this virus may exert its effect, whether it is a direct or an indirect one.[3]

Additional information on the Beltsville studies tells that a small white turkey poult of parthenogenetic origin hatched in the Spring of 1958, matured, and produced semen containing viable spermatozoa. Semen from this male was used to inseminate small white turkey hens. Three hundred and twenty eggs were incubated, of which 175 (54.7%) were infertile.

One hundred and twenty-two poults were hatched from the 147 fertile eggs produced by the fourteen hens. These poults were relatively free of major anatomical defects and thus were able to hatch unaided. They were about equally divided with respect to numbers of males and females.[4]

Another chemical that has been used quite effectively is anesthetics, especially ether, nitrous oxide, and chloroform. Mice, anesthetized with ether, produced eggs with immediate cleavage. It is also found that in the rat, one hundred percent activation of unfertilized eggs can be caused by anesthetics.[5]

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[3] Ibid., p. 1079.

[4] M. W. Olsen, "Performance Record of Parthenogenetic Turkey Male," Science, 132 (1960), p. 1661.

[5] R. A. Beatty, Parthenogenesis and Polyploidy (Cambridge, 1957), p. 27-28.

Butyric acid has been used to activate sea urchin and starfish eggs. In yet another experiment butyric acid and a concentrated salt solution were used to treat unfertilized sea urchin eggs. The treated eggs, on being returned to sea water, went through a series of processes very similar to those occurring in normally fertilized eggs, and in a number of cases, they developed in an apparently normal manner.[6]

Hypertonic and hypotonic solutions have also been tested with some success in lower animals, but very little in mammals. In one experiment, eggs of a virgin female rabbit were transplanted into the Fallopian tube of another host female, after being treated in a hypertonic saline solution. The host female was activated by what was supposed to be a sterile male. Two females, supposedly parthenogenones, developed. Because of genetic principles, this was accepted by some, but others question the sterility of the male. Another case of two parthenogenones was reported where the experimental conditions involved no males. From a donor female, eighteen ova were recovered and subjected to hypotonic salts, followed by serum, and then hypertonic salts. These over were then transferred to a host female. Two young were born. One was sterile, the other was fertile.[7]

In another unsuccessful experiment, virgin rabbit eggs were treated in a hypertonic salt solution and cultured in vitro, but none of these developed after being transferred to a host female.[8]

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[6]A. Monroy, "Fertilization of the Egg," Scientific American, 183 (1950), p. 47.

[7]R. A. Beatty, Parthenogenesis and Polyploidy (Cambridge, 1957), p. 41.

[8]A. Monroy, "Fertilization of the Egg," Scientific American, 183 (1950), p. 47.

The discovery of these methods of parthenogenesis has led biologists to believe that some day they might find a solution to the problem of what the actual stimuli for fertilization is. The results, as shown in this report, have been far inferior to what was expected. The study of these methods have made possible many approaches to the problem, but as one can see, it has brought up more questions than it has answered. We must always keep in mind that the sperm is not merely an activator, but also carries with it a set of hereditary characters, and in addition, the sperm is not ordinary, in that it will not penetrate any egg, but must have an egg of a particular species of animal.

CHAPTER V

SUMMARY

It is hoped that this paper will serve as a progress report on parthenogenetic development, and bring together material available on experimental parthenogenesis.

The experiments on artificial parthenogenesis have removed one mystical element from the problem of the initiation of development of the egg by showing that, in many cases, relatively simple chemical or physical agents can be employed in place of the complex living spermatozoan. Some of these agents include treatment of the eggs by pricking with a needle, temperature shocks, and stimulation by different chemicals.

Although parthenogenesis appears to be the exclusive mode of reproduction in certain species, it most often occurs along with regular reproduction. The recent discovery of a type of sexual reproduction in bacteria, and also in viruses at the borderline between living and non-living matter, emphasized the importance of a mechanism for the exchange of genes between individuals. Without this ability the potential evolution of a species is very limited.

As is evident, in higher animals, parthenogenesis is a deviation from the normal course of development. These parthenogenetic individuals usually lack vigor, and often do not survive to hatching, or in the case of mammals, until birth. If they do survive, they probably will not be able to produce offspring.

It is clear that we are barely on the threshold of experimentation in artificial activation and mammalian parthenogenesis. The mature, unfertilized mammalian egg is a short-lived, delicately balanced organism, and we are far from knowing the elements involved in this balanced system. It may be that in certain mammalian species the contributions of the spermatozoan are so essential that duplication of the process of fertilization in the absence of sperm is impossible. This appears not to be true of the rabbit and is probably not true of the sheep. Only further experimentation will decide the issue.

What is the use of investigating mammalian parthenogenesis? One obvious answer of course is that whatever contributes to our knowledge of the biology of reproduction is worthwhile. Another is that a parthenogenetic offspring contains only maternal genes. Furthermore, it is well on the way to complete genetic homogeneity, and the successive self-reproduction of its female descendants should lead in a few generations to a degree of genetic uniformity which in normal sexual reproduction would be attainable only after many generations of inbreeding. A successful method of parthenogenesis would therefore be very useful for breeding desirable qualities in animals, since such characteristics as high milk yield in dairy cattle, superior wool in sheep and high meat quality in beef and swine are controlled by the genes. By selecting high-quality females and reproducing the desired genes by successive parthenogenesis of the right eggs, it would be possible to get superior animals comparatively quickly. Moreover, since this method yields mainly females, it could be used to multiply the number of milk cows.

Eventually, by a combination of some of the techniques that have been described, a new type of animal breeding may be attained. We may

be able to obtain large numbers of eggs from superior animals by making them superovulate, activate these eggs by parthenogenetic means, and then transfer the eggs to ordinary females that would serve merely to develop and bear the young. But, as can be seen, it will take long years of experimentation to arrive at this goal.

Extensive investigations by many workers show that in practically all the main groups of animals normal development can be obtained by artificial activation of the unfertilized eggs, though methods of treatment may vary considerably from one species to another. From this it may be concluded that all species of animals are capable of development by parthenogenesis, either experimentally or naturally.

Could this be just one example???

> A 30-year-old English mother claims to have produced a daughter by virgin birth and science admits her claim merits serious consideration.
> Writing in the British medical journal, The Lancet, Dr. Stanley Balfour-Lynn says, "This mother's claim must not only be considered seriously, but it must also be admitted that we have been unable to disprove it."
> In response to a newspaper publicity campaign, Mrs. Emmimarie Jones stepped forward and claimed that her daughter Monica, 11, had no father. If true, this would be a clear-cut case of human parthenogenesis....
> Dr. Balfour-Lynn conducted a rigorous set of tests, including blood tests and skin grafts. Nothing was found in the child that could have come from anyone but her mother.
> Parthenogenesis has been claimed for guppies, rabbits, cats, mice, and other animals. Some biologists estimate it might occur in one woman in 1,600,000.[1]

Since this claim of human parthenogenesis has not been proved, or disproved, the question will probably remain unanswered. There is still a great need for much investigation in the field of mammalian parthenogenesis.

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[1] "Virgin Birth in Britain," Science Digest, 40 (1956), p. 54.

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