Artificial Insemination of Birds by the Intraperitoneal Route.—A Study in Sex Physiology of Pigeons and Fowls with reports upon a Modified Technique of Semen Collection, and a New Technique of Insemination, and Observations on the Spermatozoa in the Genital Organs of the Fowl Hen.

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FIRST CHAPTER.

INTRODUCTION.

A. The Historical Background.

Spallanzani (1780,1785) introduced artificial insemination to the scientific world with his records of results in dogs. The main contribution on the application of the method in breeding of farm animals was by Professor E. E. Ivanov of Moscow, who published many articles from 1899 to 1930. He was responsible for the artificial breeding of equines, which was practised successfully in the studs of the Czar, and in 1923 he was placed in charge of the Institute of Animal Breeding founded in 1919. Although the work was largely academic, results of practical importance were obtained, e.g.—

(1) protection of the sire from the risks of service;

(2) increase of the possible number of progeny of the sire;

(3) production of progeny from parents incompatible in respect of copulation.

In recent years very rapid advances have been made in the methods of artificial breeding. This is generally attributed to the economic stimulus, which in Russia for instance, at a time of great shortage of sires and minimal private ownership, led to achievements so outstanding that a world-wide interest was created (Kusnetsowa, Milovanov, Neumann, Nagaev and Skatkin, 1932).

In advanced communities artificial breeding methods have found a permanent place in intensive dairy farming, (Green, Winters and Comstock, 1944) though the technique is at present not suitable for general use where labour is scarce and expensive and where the average quality of farm stock is good or fair. (Quinlan, 1936; Falkiner, Kelly, Granger and Gunn, 1942).

B. The Problems of Artificial Breeding.

Workers on artificial insemination are confronted with difficulties particularly dependent on two factors, viz.—

(1) The genotypical superiority of the sire; (*a*) in respect of all the desired qualities, and (*b*) in the absence of recessive harmful qualities.

(2) The extra expense involved as reflected in the cost of production per animal unit.

Where the genotypical superiority is considerable, the benefits justify higher costs, and in such circumstances all the females are suitable for mating. There is then no danger of producing a harmful effect on the flock or herd as a whole. Where superiority of the genotype is not fully proved in the sire, or is confined to particular qualities as in highly bred stud stock, the sire is only mated to selected females and is less widely useful. Under these conditions the method may be of great advantage for the earley progeny-testing of young males.

If the extra cost is reduced, either by the simplification of technique or by economical use of labour and by greater technical skill, the scope of artificial insemination will be increased.

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Research is consequently at present aiming at the improvement of technique for : —

(1) *Transportation of Semen.*—The further semen can be transported successfully, the larger the potential area in which suitable females in heat may be fertilized by semen from a given sire.

(2) *Insemination.*—A high standard of technical efficiency is essential for the actual insemination. Simplification of the technique as is possible in poultry husbandry even at present, will mean that the work can be done by operators having less technical skill.

The economic value of poultry production has already led to the practical use of artificial insemination in the fowl (Bonnier and Trullson, 1939). The possibility has been visualized, that with the present methods of artificial breeding of poultry, 14,000 chicks could be obtained in one season from a single cock (*Bonadonna,* 1939; *Burrows and Quinn,* 1939). The productive life of the fowl cock is at its maximum in his first season (9-15 months) and is rapidly reduced after a year (Munro, 1938) so that the use of an outstanding, proved sire, to a greater extent than is naturally possible, may have considerable value.

Overproduction of spermatozoa takes place in birds as in most classes of animals. Only a small proportion of the male cells deposited in the vagina or cloaca at coitus ever reach the upper end of the oviduct, where ova are subject to impregnation (Hammond, 1940).

Artificial insemination introduces some conditions unfavourable to the chances of fertilization such as exposure of the semen to air and contact between spermatozoa and the walls of receptacles. However by depositing the semen of the fowl as far forward as possible in the vagina (Burrows and Quinn, 1939) or in the uterus (Munro, 1938), results have been obtained showing advantages over natural mating. (Bonnier and Trullson, 1939; Warren and Gish, 1943).

C. The Objects of the Present Work.

When these facts were considered it appeared that the chances for success of artificial insemination in the bird might be improved by depositing the spermatozoa as near as possible to the locality where the penetration of ova occurs. This process of impregnation, in birds as in mammals, is possible only in the vicinity of the ovary (Hammond, 1940).

Consequently it must take place in birds very soon after ovulation and probably in the entrance to the oviduct. The phenomenon of oestrus is not found in the bird and the season of sexual activity in the domestic pigeon and fowl extends throughout the year, with the exception of the period of moulting.

As pigeons could easily be obtained and since the time of ovulation in these birds can also be predicted within very narrow limits of time (Riddle and Behre, 1921; Owen, 1941) this species was considered suitable for carrying out the initial attempts to introduce semen via the peritoneal cavity to the region of the ovary. If it could be proved that the passage of spermatozoa through the pigeon oviduct was not a prerequisite to successful fertilization, then trials with intraperitoneal insemination could be made in the fowl. Ovulation in the domestic hen is a process repeated at short intervals over long periods of time and ova may continue to be fertilized several days after separation from the male, which makes the hen a very suitable experimental subject for studies on artificial insemination. The high natural resistance of birds to infection with pyogenic bacteria was also considered a favourable factor.

If such fertilization could be effected in the fowl hen, investigations into the fate of spermatozoa might throw further light on some of the interesting and unexplained phenomena in avian sex physiology, viz.: the long duration of fertility after ciitus without any apparent spermatotheca or localities of concentrated stored sperm *in vivo;* and the replacement of fertile stored sperm by fresh sperm from a more recent coitus.

The objects of this thesis are:—

(1) To present a comprehensive review of the literature relevant to artificial insemination of birds and the storage of spermatozoa *in vivo* in the oviduct of the hen.

(2) To describe a simplified method of semen collection in the pigeon and fowl.

(3) To describe a technique by which ova of birds could be fertilized with semen discharged in the region of the ovary.

(4) To present data indicating the value of the above new technique.

(5) To examine some biological relations between spermatozoa from different males simultaneously present in the oviduct of the fowl hen.

(6) To describe the determination of the site and method of storage of spermatozoa in the fertilized fowl and how this was influenced by the new technique of artificial insemination.

SECOND CHAPTER.

REVIEW OF THE LITERATURE.

Professor E. E. Ivanov (1913) studied artificial insemination in the fowl using semen from the vasa deferentia of killed males and reported on the nature of fertility in the hen. Payne (1914) also performed artificial insemination in the fowl and followed up the activity of the spermatozoa in the oviduct of the fertile hens kept separated from the cock. Since then numerous authors have reported results from work done on sex-physiology in fowls and other birds (Amantea, 1922; Ishikawa, 1930 ; Timyakov, 1933; Grecka, 1935; Quinn and Burrows, 1936; Burrows and Quinn, 1937, 1939; Milovanov, 1938, Griffini, 1939; Bonnier and Trullson,1939 ; Bonadonna, 1939; Parker, MacKenzie and Kempster, 1940, 1942; Black and Scorgie, 1942; Gracewski and Scott, 1943). This research was noticeably stimulated in 1935 when a cheap and harmless method of collecting semen easily and directly from the cock, was discovered by Burrows and Quinn (1935, 1937, 1939); but there is no doubt that the progress made in the study of artificial insemination of farm animals generally (Ivanov, 1900 to 1930; Kusnetsowa, Milovanov, Neumann, Nagaev and Skatkin, 1932; Walton, 1933, 1936, 1938, etc.,) was the main factor responsible for the great interest in this branch of poultry science, noted from recent publications. The developments of technique have thus as a rule been based on experience with other farm livestock, and the results have been reported in fairly recent publications.

The physiology of reproduction includes a sequence of events, which may be classified in the following stages: —

1. The production of spermatozoa and ova, coitus and ejaculation.

2. The entry of the spermatozoa into the organs of the female.

3. Viability and migration of the spermatozoa and ova in the genital tract and adjoining organs of the female.

. 4. Impregnation of the ovum and the union of the male and female gametes.

5. The migration and development of the zygote, embryo and new-born young.

The following review of literature relevant to fertilization, egg-production, incubation and hatching of fowl eggs and pigeon eggs, and to semen collection and sperm physiology in the fowl and pigeon will be considered in the sequence of this classification, with particular attention accorded to artificial methods of insemination and *the route of insemination.*

Much of the knowledge is based on mammalian research and where necessary, this will be mentioned first in each case.

FIRST STAGE.—THE PRODUCTION OF SPERMATOZOA AND OVA, COITUS AND EJACULATION.

The anatomy and physiology of avian reproductive organs have been studied by Bradley (1915, 1928), Kaupp (1915), Heuser (1916), Craft, McElroy and Penquite (1926), Crew (1926), Penquite, Craft and Thomson (1930), Bisonette and Walund (1932), Adamstone and Card (1934), Burrows and Quinn(1937), Gray, (1937), Parker, MacKenzie and Kempster (1940, 1942), Parker and McSpadden (1943 a and b), Wheeler and Andrews (1943) and many others. Comprehensive reviews on various aspects exist, (Domm, 1939; Moore, 1939; Parker, MacKenzie and Kempster, 1942; Anderson, 1945).

A. *Natural Copulation.*

In nature the discharge of semen from the male organs into the female genital tract involves physical activity of both male and female, The part played by the female includes the active process of everting the vaginal mucosa, and has been referred to as " receptivity " (Lamoreux, 1940; MacKenzie and Kempster, 1942). In most birds the duration of contact between male and female during coitus is only a matter of seconds. The male copulatory organ is formed by a portion of the cloacal mucosa containing the terminal ends of the vasa deferentia or " bulbous ducts " (Burrows and Quinn, 1937; Parker, *e. a.* 1942) or the posterior ends of the vasa deferentia. Semen is deposited on the everted vaginal mucous membrane, a portion often entering the cloaca (Payne, 1914; Jull and Quinn, 1931). The fowl cock " treads " up to 30 or 40 times a day in the breeding season (Heuser, 1918; Martin and Anderson, 1918; Penquite *e.a.* 1930); but 14 per cent. of copulations may not be accompanied by ejaculation (Parker *e.a.,* 1940). " Libido " in the fowl cock is affected by preferential mating (Heuser, 1916; Upp, 1928), the number of females present (Phillips, 1918; Martin and Anderson, 1918) and the time of the day (Parker *e.a.* 1940) showing an increase in the late afternoon. The hen is receptive to copulation much less frequently than the cock will tread.

Both production of spermatozoa and the distribution of sperm, between the different copulations are factors determining the value of a cock. Munro (1938 *b*) found that a minimum of 1×10^8 sperm per insemination was required for good

fertility and Parker *e.a.* (1942) estimated that if each ejaculation were limited to this number, cocks would be capable of fertilizing 17 to 24 females per day, but as 50 per cent of ejaculations collected after rest contained 5×10^8 and 20 per cent. less than 1×10^8 it seemed that under natural conditions only a few hens could be fertilized properly by one male in one day..

The ejaculate of the fowl cock has a density of 250,000 to 10,200,000 spermatozoa per cubic millimeter, with an average of 3,200,000. The total volume of semen per collection after 24 hours rest is $0 \cdot 25$ to $2 \cdot 25$ ml. with an average of almost $1 \cdot 0$ ml. (Burrows and Quinn, 1935, 1937, 1939). The volume that may be obtained from one cock in one day at repeated collections may reach $4 \cdot 5$ ml. and it varies according to the breed and age of the bird, being highest in cockerels of the light breeds. The volume of the semen, and the total number of spermatozoa produced per ejaculation, and the period of maximum life duration of the sperm-cells *in vitro* all show a seasonal decline from highest in late winter to lowest in midsummer (Munro, 1938; Burrows and Titus, 1939; Wheeler and Andrews, 1943). Penquite *e.a.* (1930) reported a reduction in amount of fowl semen ejaculated at successive matings on the same day and Warren and Gish (1943) found that the second collection of a cock's semen on the same day yielded on an average a quarter to a third less semen than the first, whether it was taken within half an hour or eight hours after the first. According to Jones and Lamoreux (1942) there is a direct genetic correlation between the semen production in the male fowl and the egg-production of his sisters and offspring; but comb and wattle size, or sexual activity, are no indication of his semen producing qualities (Searcy and Andrews, 1937).

B. *Artificial Semen Collection.*

The aims of artificial insemination as mentioned in the introductory chapter have to be recapitulated with particular reference to the fowl:—

(1) *Disease control.* Infections transmissible by coitus in the fowl are few, e.g. " vent gleet ", but verminosis, coccidiosis, fowl typhoid etc. can be transmitted by contact in the same pen, and would be less likely to infect the males isolated for artificial semen collection.

(2) *Subdivision of the ejaculate* with and without the aid of diluents in order to increase the number of hens fertilized from one male was practised by Burrows and Quinn (1939), Bonnier and Trullson (1939), Griffini (1939), Black and Scorgie (1941) and Gracewski and Scott (1943).

Most of the information available, is however, based on the work with sheep and cattle (Kusnetsowa *et al*, 1932; Walton, 1933; Goetze, 1933; Phillips, 1935; Quinlan, 1936; Hammond, 1940; Milovanov, 1940; Quinlan *et al.*, 1943). The data which have been collected, have led to acceptance of the following facts:—

(*a*) In all animals there exists a minimal limit to the numbers of spermatozoa required for fertilization (Walton, 1927; Munro, 1938). Munro (1938) showed that the number of fertile eggs produced by a hen was adversely affected when the number of spermatozoa introduced was about a hundred million (100,000,000) or less; and that no eggs were fertile when this number was below one million. This minimal requirement was a hundred times higher than in the case of the rabbit (Walton, 1927) and Munro suggested that this difference might be due to the comparatively large size and heavily ridged internal surface of the hen's oviduct. Burrows and Quinn

(1938) determined the smallest fully effective dose of pure fowl semen to be 0·05 c.c. for normal cocks in the breeding season, whilst Bonnier and Trullson (1939) maintained a high degree of fertility with only 0·05 c.c. of a 10 per cent. dilution of fowl semen. They did not mention the concentration of sperm in these cases. Bonadonna (1939) expressed the opinion that the available data permitted the statement that 15,000 chicks might be obtained from a single cock in one season.

(b) The lower the concentration of spermatozoa in the semen the larger is the portion of the ejaculate required for an insemination with optimum chances of success (Munro, 1938; Hammond, 1940).

(c) Sperm cells are easily damaged by physical and chemical changes of their environs e.g. dilution and temperature (Walton, 1933; Milovanov, 1940; Quinn et al., 1943). The technique of dilution will be dealt with under the heading " Storage of semen ", as it applies when the semen is in artificial containers outside the animal body.

(3) *Transport or transfer of the semen* is also a matter for consideration under " storage " as it depends on the vitality of the cells *in vitro* and the ways and means available for protection and also on the time factor. (Distance and speed of transportation). Some cases of effective transportation of ram and bull semen over great distances are on record (Lambert and McKenzie, 1940; Walton and Prawochensky, 1936; Phillips, Scott and Gildow, 1938; Terril and Gildow, 1938) and in Russia, the homing-pigeon, motorcar and aeroplane have served as a means of conveyance.

(4) *Storage of semen* is therefore the important aspects of artificial breeding, the degree of success being directly dependent on the following factors : —

    (a) Specific qualities of the type of semen.

    (b) The methods of collection.

    (c) The materials of contact.

    (d) The temperature.

    (e) The pH. These will be considered in the sequence given.

(a) The species differences are shortly summarized in the following table (Table 1), based on information given by Walton (1933), Milovanov (1940), Hammond (1940) and Starke (1943). The large volume of accessory secretions present in semen from the boar and stallion makes the effective storage period of short duration. Ram and bull semen, can, however, be stored over a period of a week or more when favourable conditions can be provided, and can then still be used successfully for insemination.

    The keeping qualities of fowl semen have consistently been found to be relatively poor, notwithstanding the total absence of accessory secretions in the fowl product, and the higher concentration of sperm in it. Warren and Gish (1943) found a drop in fertility from 73 per cent. for fresh semen to 66 per cent. for five-hour-old semen, and to 6 per cent. for 24-hour-old semen, when stored at room temperature (50-60°F.). They report total loss of

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fertilizing power in semen subjected to 32° F. and 80° F. for seven hours. Shaffner, Henderson and Card (1941) succeeded in obtaining a single chicken from momentarily frozen sperm. (– 6°C.).

TABLE 1.

The Species Differences of the Semen and the Ejaculate in Domestic Animals

Line	Species.	Average Volume of Ejaculate.	Average Density of Spermatozoa per Cubic Millimetre.	Average Hydrogen-ion Concentration.	Average Maximum Duration of Storage Period *in vitro*.	Minimum Dose of Diluted or Pure Semen required for Optimum Fertility.	Average Number of Females Inseminated from One Ejaculate.	Average Number of Progeny per Insemination.	Average Number of Progeny per Ejaculate.
	Column (1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
a	Pig.......	ml. 200·0	100,000	7·00	6 hours	ml. 100·0	2	9	18
b	Horse....	100·0	60,000	7·40	12 hours	20·0	*(a)* 8	1	*(a)* 8
c	Bovine...	4·0	800,000	7·00	3 days	*(a)* 1·0	*(a)* 10	1	*(a)* 10
d	Sheep....	1·0	1,000,000	6·10	5 days	0·1	*(a)* 30	1	*(a)* 30
e	Fowl.....	*(d)* 1·0	*(g)* 3,000,000	*(c)* *(g)* 7·04–7·27	*(b)* 8 hours	*(d)* 0·1 *(a)* 0·1	*(d)* 10 *(h)* (50–100)	8 ?	80 Chicks ?
f	Turkey...	*(d)* 0·3	?	?	?	*(d)* 0·05	*(f)* 6	8	48
g	Pigeon....	*(e)* 0·01	?	?	?	*(e)* 0·01	*(e)* 1	2	2

References:—
(a) With diluted semen (1 : 1 to 1 : 3).
(b) Warren and Gish, 1943.
(c) Wheeler and Andrews, 1943.
(d) Burrows and Quinn, 1937, 1939.
(e) Owen, 1941.
(f) Burrows and Marsden, 1938.
(g) Parker, MacKenzie and Kempster, 1942.
(h) Bonnier and Trullson, 1939.

(b) Methods of semen collection.—The earlier primitive methods of obtaining semen were the draining of vasa deferentia in freshly killed males (Ivanov, 1913; Hammond and Ashdell, 1926; Walton, 1938) and collection from the vagina immediately after coitus. e.g. the sponge method in equines (Ivanov, 1923; Walton, 1913) and the glass spoon or pipette inserted into the cloaca of the hen

(Payne, 1914; Craft, McElroy and Penquite, 1926; Jull and Quinn, 1931). The dog may be made to ejaculate by manual stimulation of the penis (Spallanzani, 1780, 1785; Starke, 1943).

These methods have since been discarded in farm animals in order to collect a cleaner and more concentrated ejaculate, though laboratory animals are often still put to death for the purpose of obtaining sterile experimental material (Hammond and Asdell, 1926; Walton, 1930; Mimura, 1939). Sperm may be taken from the epididymis by a syringe and metal needle, but the metalic contact is detrimental to the sperm cells (Walton, 1933). In domestic animals the artificial vagina has proved to be the method of choice (Ivanov, 1930; Milovanov, 1938; Quinlan, 1936.) Other methods known at present are: the ejaculation of the bull by massage of the ampulae per rectum (Miller and Evans, 1934); electro-ejaculation in the ram (Gunn, 1936; Quinlan, *e.a.* 1941, 1943; Hammond, 1940); the abdominal massage method for the cock (Burrows and Quinn, 1935); and injections of pilocarpine for the dog (Moore, 1941).

All the methods applied to the fowl are given in the following list:—

(i) Draining of the vasa deferentia of killed males.

(ii) Using both hens and cocks together.—

   (*a*) securing fluid from the cloaca immediately after coitus using a glass spoon or pipette (Payne, 1914; Craft, McElroy and Penquite, 1926; Jull and Quinn, 1931);

   (*b*) placing a container between the cock and hen during mating (Amantea, 1922; Dunn, 1927; Warren and Kilpatrick, 1929; Hutt, 1929);

   (*c*) attaching an artificial cloaca of wire and animal membrane to the hen (Ishikawa, 1930) or a rubber instrument (Timjakov's " spermaceptor cloacale ", Adamstone and Card, 1934; Grecka, 1935; Milovanov, 1938; Kuzjmima, 1933).

   (*d*) attaching a receptable to the cock when mating (Parker, 1939).

(iii) Using live cocks alone:—

   (*a*) collecting semen by means of electrical stimulation (Serebrowski and Sokolowski, 1935; Letard and Tinet, 1937) (80 volt, 3-4 secs., 1-2 secs. intervals; Positive:—skin, sacrum; Negative:—water, beak).

   (*b*) stimulating the abdomen and vent by manipulation, to bring about ejaculation (Burrows and Quinn, 1935).

   (*c*) " milking " of the bulbous ducts or vasa deferentia after stimulation as in (*b*) (Burrows and Quinn, 1937, 1939).

It is now generally agreed that artificial collection of fowl semen in practice, is best done by the latest method of Burrows and Quinn i.e. (iii) (*c*). This method was applied to Turkeys (Burrows and Quinn, 1937; Burrows and Marsden, 1938) and adapted to Ducks, Peacocks, Finches and Canaries (Bonadonna, 1939) as well as to pigeons and doves (Owen, 1941).

According to McKenzie, Miller and Bauguess (1938), the duration of motility of sperm in semen of the boar stored *in vitro*, was reduced when the interval between successive collections from one male was decreased from 72 hours to twelve (12) hours.

No reports on similar data in the fowl were seen but successive collections at intervals of one hour showed a decrease in amount or volume of ejaculate (Sampson and Warren, 1929; Warren and Gish, 1943) as well as in the density of the semen obtained (Parker, McKenzie and Kempster, 1942). The latter found an increase in the alkalinity contrary to the experience of Anderson (1941) and Williams (1939) with the second ejaculate in normal bulls.

(*c*) Materials of contact: i.e. the material of receptacles and the diluents for semen (diluters, dilutors).

(i) Semen receptacles.—In the practice of semen collection the commonly used materials which have a harmful effect on the spermatozoa are rubber and metal, whilst the contact with air and deposits of dirt or salts on the instruments is recognised as unfavourable to sperm life. The harmless materials are sterile, dry, hard glass, free from traces of chemicals and the pure paraffins e.g. liquid paraffin and white vaseline (petrolatum).

The " Cambridge " method of sperm storage consists of placing fresh semen immediately in a clean glass receptacle and covering it with a layer of sterile liquid paraffin closing the mouth with a stopper or cottonwool plug to keep out dust particles (Walton, 1933). In contact with air semen takes up O_2 and gives off CO_2 in the process of respiration, which increases sperm activity and reduces their life duration, (Hammond, 1940). Rubber parts and lubricants are essential for the construction of artificial vaginae, but liquid paraffin may be used for preventing contact between spermatozoa and rubber (Bernstein, 1933; Walton, 1933, 1938).

Gelatin capsules coated with paraffin wax were found promising as semen receptacles (Smirnov, 1938) and cocoa-nut-butter was found useful for making containers in some instances, but no record appears to exist of attempts to exclude the effect of metal surfaces on semen in contact with them, by coating the metal parts with paraffin wax.

Fowl semen has been kept for short periods in an adapted glass funnel of which the stem was occluded with a plug of vaseline (Burrows and Quinn, 1939, 1937, 1935). The semen was transferred and inseminated by means of an all-glass insulin syringe or a glass pipette. Burrows and Quinn, (1939) reported that semen soiled with fæces was of little value. Pigeon semen was colected by Owen (1941) with a small glass pipette.

(ii) Diluents and Media (diluters, dilutors).—Semen is best stored in the pure state according to most records available (Weber, 1936; Komarov and Gladcinova, 1937; Winburg, 1939; Green, Winters and Comstock, 1942), but Kusnetsowa *e.a.* (1932) claimed that the extension of sperm life was one of the aims

of dilution, which Milovanov (1940) explained was to be brought about by improving the environs of the spermatozoa. The views of Milovanov (1940) are illustrated clearly in graph A. The character of the curves and the presence of a toxic point were proof that favourable conditions were obtained as the result of a combination between diluent and natural environment of sperm in the semen. Ivanof used isotonic salt

GRAPH A.—An illustration of the effect of good and unsuitable media for dilution of semen on the viability of the spermatozoa in the artificial media at different degrees of dilution.

(Graph after Milovanov, 1940).
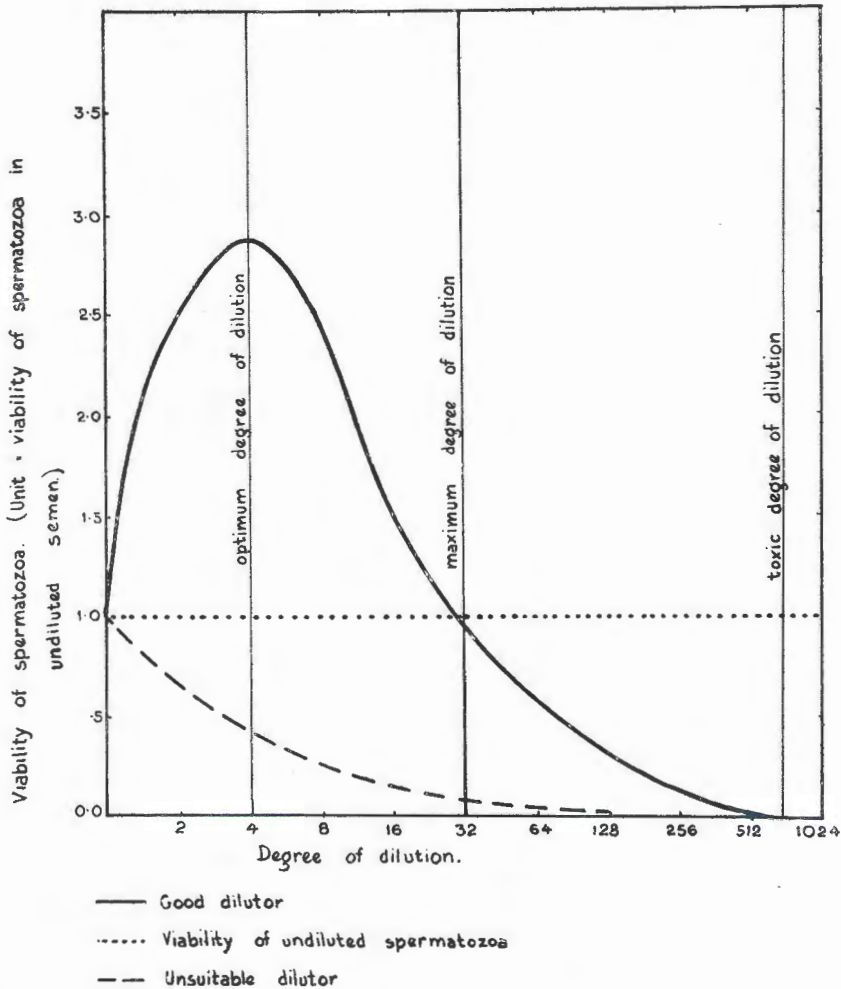
Graph A shows an arbitrary comparison of sperm viability to be found at different degrees of dilution of semen in a good diluent and in an unsuitable diluent. In the case of the former, the viability of the spermatozoa is improved by each drop of diluent added to the semen until an optimum degree of dilution, for the particular semen and diluent is reached, after which sperm viability is reduced by increased dilution to the level of that found in pure semen and eventually down to the level where all spermatozoa die (toxic point).

solutions. Redenz (1926) discovered the useful action of glucose in reduction of electrolytes like Ringer's and Tyrode solution. Kusnetsowa *e.a.* (1932) developed the gluco-phosphates. Lardy and Phillips (1939) recommended a yolk-phosphate diluent. Serum may be used after inactivation for one hour at 60° C. (Bernstein, 1933; Grodzinski and Marchlewski, 1935). Yolk-citrate has been used successfully (Salisbury, *e.a.* 1941).

In the fowl the earlier diluents were made from thin egg albumen and isotonic salines (Nikitina, 1932; Griffini, 1938;). Munro (1938) demonstrated the detrimental effects of some diluents on fowl sperm as compared with " sperm serum " and also the temperature-medium interaction varying so markedly from mammalian sperm physiology. In " sperm serum ", blood serum, thin egg-white and in shell gland fluid motility of fowl sperm was found to be supported at temperatures between 75° F. and 105° F., but in saline, Tyrode or Ringer's solution as well as in fluid from the infundibulum and albumen secreting portion, the sperms were immobilized at 105° F. though the change was reversible and they regained motility when the temperature was lowered. According to Loeb and Bancroft (1912) fowl sperm showed morphological changes (cytoplasm arrangement) when kept at body temperature in albumen after a few hours. In Ringer's solution these changes, which they believed to be developmental, did not occur even after 48 hours. Warren and Kilpatrick (1929) found that fresh sperm in saline or albumen at 100°F., unlike the spermatozoa which they observed in the oviduct, retained their flagella as long as they lived, i.e. more than 48 hours.

(*d*) The Temperature of Storage.—Quinlan *e.a.* (1943) retained the motility potential of ram sperm for 100 days by storage at a temperature between 2° C. and 4° C. The fertilizing power was under these conditions only maintained for 72 hours (Quinlan *e.a.,* 1941). Cooling of semen had to be gradual (Milovanov, 1940) e.g. 5° C. per two hours for storage at 1° C., and 5° C. per 20 minutes for storage at 5° C. Heating should be rapid when used for insemination (Salisbury, 1942).

Fowl semen has the higher ejaculation temperature of the bird. Warren and Gish (1943) found that 50° F. was the best storage temperature and that the fertilizing power was destroyed by keeping the semen at 32° F. and at 80° F. for seven (7) hours. The duration of fertilizing capacity of stored fowl semen has been found to be short (Burrows and Quinn, 1937). The production of a single chick from momentarily frozen sperm by Shaffner, Henderson and Card (1941) has been mentioned. According to Grodzinski and Marchlewski (1935) the optimum storage temperature for the retention of motility of fowl sperm stored in albumen medium was 16° C. and in other media 2° C. The medium temperature interaction on sperm of the fowl has been mentioned (Munro, 1938). Winburg (1941) found fowl spermatozoa to resist freezing at – 15° C. for 1 to 10 minutes freezing, depending on the rapidity of freezing and rewarming. Sperm in semen stored

at 20° C. however survived the longest. This worker reported a motility threshhold at 6° C. i.e. at this temperature sperm of the fowl become immotile on cooling and recovered their motility on rewarming if still viable. Wheeler and Andrews (1943) obtained survival of potential motility of spermatozoa of the fowl for fifteen (15) days at 2-3° C.

(*e*) The Hydrogen-ion concentration.—According to Grodzinski and Marchlewski (1935), the optimum range of pH value for stored fowl semen was 7·2–8·0. Fresh semen had an average pH of 7·04 or 7·27 (Buckner and Martin, 1929; Zagami, 1937; Parker, McKenzie and Kempster, 1940, 1942; Wheeler and Andrews, 1943). The sealing off of semen samples by a layer of liquid paraffin has the effect of increasing the CO_2 tension in the semen with a rise in pH and with the reduction of activity. Redenz (1927) showed that motility under anaerobic conditions was supported by glycolysis (i.e. not respiration but followed by lactic acid formation). The low pH of the contents of the epididymides, has been mentioned as a factor for conservation of sperm energy.

C. *Evaluation of Semen Samples.*

Some of the techniques for estimating the quality of semen samples have been used in the study of fowl semen.

The *volume* of fowl semen obtained per artificial collection was reported as 0·10 ml. to 1·10 ml., average: 0·40 ml. (Burrows and Quinn, 1937), 0·10 ml. to 1·25 ml. average 0·40 ml. (Griffini 1938), 0·42 ml. to 0·30 ml., (Burrows and Titus, 1939) and 0·25 – 1·50 ml. average: 0·88 ml. (Parker *e.a.*, 1942). The *colour* has been described as white or milky (Raimo, 1943), and the *viscosity* given as thick or watery for good and poor semen respectively.

Several routine tests provide a basis for evaluation:—

(1) *Microscopical examination for motility:*

Hanging drop slides have been used (Raimo, 1943) and specimens handled with a round-ended glass rod as recommended by Walton (1933) to avoid the effect of alkali, given off by some glass pipettes. The motility scores of Roemmele (1927) i.e. " 1 " for good to " 6 " for non-motile sperm and of Kusnetsowa *et al* (1932) and Walton (1933) i.e. " 5 " to " 1 " have now generally been replaced by that of Milovanov (1940), who recognised that the progressive vigorous motility was the all important criterion and thus indicated motility as decimals of a unit representing 100 per cent. progressive motility (Raimo, 1943). In the fowl a correlaton between motility of sperm and fertilizing power of the semen was established by Parker, McKenzie and Kempster (1942).

In addition to—

(*a*) the percentage,

(*b*) type and

(*c*) energy of motility, some workers have used

(*d*) the duration of motility *in vivo* and

(*e*) *in vitro* with observation on: —
>   (i) the resistance to sudden drops of temperature (Quinlan, 1944);
>   (ii) the resistance to immobilisation by dilution with isotonic NaCl solutions (Milovanov, 1940);
>   (iii) the resistance to immobilization by changes in pH of the medium;

but this has not been reported for the fowl.

The maintenance of potential motility *in vivo* (*d*) in the female genital tract has been examined by Yochem (1929, 1930), and Quinlan *e.a.* (1932) and reports on the fowl from this asppect exist (Payne, 1914; Warren and Kilpatrick, 1929; Walton and Wetham, 1933; van Drimmelen, 1945b, 1946a).

(2) *Estimation of the Density of Semen.*

(*a*) The hæmocytometer counts for estimating the density of fowl semen e.g. Payne (1914): 1,920,000-5,470,000 per m.m.^{3}; Amantea (1922): 4,000,000 per m.m.^{3}; Craft *e.a.* (1926): 2,000-4,000,000 per m.m.^{3}; Hutt (1929): 18,700-8,864,000 average 4 million per m.m.^{3}; Ishikawa (1930): 115,000-182,000, average 141,300 per m.m.^{3} Sampson and Warren (1939): 40,000-1,750,000, average 1,064,000 per m.m.^{3}; Parker, McKenzie and Kempster (1940): nil to 10,030,000, average 2,340,000 per m.m.^{3}; Raimo (1943): 1,270,000-3,496,600, average 2,070,400 per m.m.^{3}; and Wheeler and Andrews (1943): 250,000 to 10,200,000, average 3,200,000 per m.m.^{3}, show the marked variation in results obtained with this instrument. The laborious and time consuming technique, though suitable for sterility work in the human being (Hotchkiss, 1936; Pollock and Joel, 1939) is in animals usually replaced by an estimate (Starke, 1943; Comstock, Green, Winters and Nordskog, 1943).

(*b*) Estimate from microscopical examination of dry semen films (Sampson and Warren, 1939).

(*c*) Burbank (1935) tried quantitative standardization of sperm suspensions by means of opacity. Comstock *e.a.* (1943) showed this to be more accurate than hæmocytometer counts in the ram but no record was found of the method having been applied to fowl semen.

(*d*) Shaffner and Andrews (1943) measured density by centrifugation of semen (fowl and bovine) in small capillary tubes.

(3) *Microscopical examination of sperm morphology in hanging drop slides, and stained and unstained dry films of semen.*

(*a*) The determination of shape and form of the spermatozoon.—This cell was described by Bradley (1915), Adamstone and Card (1934), Parker, McKenzie and Kempster (1942) and Raimo (1943) in the fowl. The latter submits that the spermatozoon of the fowl conforms in structure to the sperm of other animals and he presents a sketch giving details (in accentuated form) of the acrosome, anterior centrosome, nucleus, para-basal corpuscle, proximal centrosome, axial filament and anterior and posterior parts of the distal centrosome. Parker *e.a.* (1942) gives the length of the head as 15μ the middle-piece as $3\cdot2\text{-}4\cdot8\mu$. Adamstone and Card (1934) describe the short recurved acrosome, the long cylindrical and arc-shaped head and the mid-piece and tail tapering to a very fine end. They particularly mention a row of highly refractile lipoid bodies arranged like a string of beads in the head of the fowl sperm along its entire length (first reported

by Guyer, 1909). These bodies were found to be divided up and scattered throughout the nucleoplasm as the cell became older. Ageing also resulted in a loss of affinity for stains, a lengthening and broadening of the head and a sinuous shape in place of the clear-cut arc-shape of fresh sperm. Abnormal fowl sperm was described and estimated by Parker e.a. (1942); the range was 3·5 per cent to 39 per cent. abnormals of eleven different types and with tail abnormalities the most common. A percentage of abnormalities higher than 15 per cent. affected fertility, the negative correlation between percentage of abnormal sperms and percentage of fertility being highly significant. Sampson and Warren (1939) mentioned this too.

(b) The cells may be measured by means of a micro-projector but no records of such measurements for the fowl are available.

(c) Staining reactions indicating the quality of spermatozoa have been reported by Adamstone and Card (1934).

(d) Abnormal contents of the semen of the fowl have not been described (e.g. leucocytes, bacteria, etc), but Fish (1924) reported on the non-sperm constituents in the semen.

(4) *Chemical examination of the semen.*

Glycolysis (the formation of acid from the metabolism of sugars) and respiration (oxygen consumption or CO_2 formation) have not been extensively studied in the fowl. The processes are apparently not expected to vary markedly from similar changes in other semen in which measurements have been recorded with : —

(a) the reduction of methylene blue (Sørensen, 1941).

(b) metabolism rate and

(c) respiration rate (Winburg, 1939).

(5) *The maintenance of potential fertilizing capacity.*

These tests are the real proof of, and the only means of control for the results of the tests mentioned, as far as the value of a given sample of semen is concerned. The duration of the period of successful storage of fertile semen is of prime importance in artificial insemination and in the fowl the maintenance of fertilizing power *in vivo* in the hen's organs is particularly important in view of the regular almost daily ovulation (Parker *e.a.* 1942; van Drimmelen, 1945 b, 1946 a.). Fertility can be maintained under various conditions: —

(a) *In vitro* : —

(i) at different temperatures: (Quinlan *e.a.* 1941; Walton, 1938; Weber, 1936; Milovanov, 1940). The good motility of fowl sperm maintained when stored *in vitro* is apparently not associated with their fertilizing capacity as Burrows and Quinn (1919) found, that when semen was stored at 4·4° C. or lower the fertilizing ability was lost in two hours or less. More data on the effect of temperature have been mentioned [see B(4) (d); temperature of storage].

(ii) in different diluents: (Walton, 1936, 1938; Razumev, 1938; Milovanov, 1940; Quinlan *e.a.* 1941. Sørensen 1941.) Fowl semen was kept with little success in artificial media: the best results being with thin egg-white (Griffini, 1938).

(iii) at various pH. levels: (Quinlan *e.a.* 1941); not recorded in the fowl.

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(b) *In vivo* in the female organs.

The duration of fertility after coitus was first studied by Barfurth, (1896) and since then by numerous authors as will be mentioned later. Although it is important in the rabbit (Hammond and Asdell, 1926) and other mammals (Quinlan, 1932) in view of the fact that ovulation may occur too late after service under certain conditions (Walton, 1933; Hammond, 1938; Lambert and McKenzie, 1940; Anderson, 1941, b; Quinlan *e.a.* 1941, 1936; Day, 1932), the fowl is specially affected by the length of life of sperm in the oviduct, because the number of eggs fertilized from one insemination of coitus is very closely associated with it (Ivanov, 1924; Crew, 1926; Warren and Kilpatrick, 1929; Walton and Wetham, 1933; Burrows and Quinn, 1938; Parker *e.a.* 1942; van Drimmelen, 1945 a and b, 1946 a).

NOTE.—*On some descriptive terms.*

It is of great importance that in all descriptions of spermatozoa the terms " Viability ", " Vitality " and " Life-duration ", shall be clearly defined in respect of motility, as well as fertilizing capacity. Motility is apparently a function of the middle-piece of the spermatozoon (Phillips, 1935) the proximal centriole being at the source of power (Poppa and Marza, 1930). Notwithstanding the experience of Burrows and Quinn (1939) to the contrary, Parker, e.a. (1942) were able to establish the correlation between motility of sperm and fertilizing power of the semen.

SECOND STAGE.—THE ENTRY OF SPERMATOZOA INTO THE BODY OF THE FEMALE.

A. *Natural Processes.*

(1) *The location of entry.*—Semen is deposited at coitus in the anterior part of the vagina in all species of domestic mammals (Hammond, 1940), except in the mare and sow in which it normally passes through the open cervix into the uterus. In the fowl hen and in the pigeon the everted vaginal mucosa receives the semen during contact with the male cloaca.

(2) *The time of Entry.*—The seasons of sexual activity are in nature the only periods during which the sperm enter the female tract.

In mammals the male organ is not permitted to enter the vagina except during the period of heat or " oestrus." Domestication has in some cases extended the periods of sexual activity or breeding seasons, but not the periods of oestrus (slightly?). Climatic and nutritional circumstances may play a role in determining these.

The phenomenon of " oestrus " is absent in the fowl although the " hatching " season is a favourable time for rearing chicks. Breeding is actually possible throughout the year with increased activity in early spring and decreased activity during the moult in late summer. The fowl hen is receptive to, (i.e. will permit,) fewer copulations than the cock, 'and whereas some hens in full production will frequently receive the cock, others, usually poor egg-producers may evade, or be neglected by the cock for several days in succession, (Lamoreux, 1940; Nalbandov and Card, 1943).

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B. *Artificial Interference.*

(1) *The Route of entry.*—Artificial insemination has the aim of placing the male cells in the female organs in a locality which will:—

  (i) enhance the chances of reaching the ovum in time;

  (ii) decrease the minimum number of spermatozoa required for optimum fertility.

The route of entry has always been sought on the principle expressed by Walton (1933); " following as closely as possible the conditions in nature, keeping in mind, that these are not necessarily the most favourable conditions; and that improvements even on the natural processes are desirable. The ways of nature are, however, preferable to methods which introduce unnecessary deviations with possible unknown influences. " Thus in the cow and ewe, by the use of special pipettes, the semen was deposited in the cervix during artificial insemination (Neuman, 1935, cit. Hammond, 1940; Quinlan, 1941). On account of the fragility of the glass instruments used for insemination, hard rubber pipettes (Kusnetsowa, 1932) and vulcanite or plastic pipettes have been used (Walton,1936, 1938). Metal inseminators are sometimes employed (Green, Winters and Comstock, 1943).

In fowls Quinn and Burrows (1936) and Burrows and Quinn (1939) recommended an all-glass insulin syringe for insemination into the artificially exposed vagina of the hen. Others have found blunt glass pipettes very suitable (Bonnier and Trullson, 1939; Black and Scorgie, 1941; Gracewski and Scott, 1943). Munro (1938) was able to discharge the semen inside the uterus, as the hen has no cervix uteri to hinder such entry (Mimura, 1939). Jull and Quinn (1931) obtained a 20 per cent. fertility from insemination simply into the cloaca.

After natural copulation Nicolaides (1934) reported 15 per cent. of infertile cases and Parker, McKenzie and Kempster (1940) found 13 per cent. Following artificial insemination a number of infertile cases were also noted e.g. by Bonnier and Trullson (1939); Quinn and Burrows, (1939); Burrows and Marsden, (1938) and Bonadonna (1939). However, by means of artificial insemination, fertility in 10 turkey hens was increased from 7·5 per cent. to 88·4 per cent., on the previous results with natural mating (Burrows and Marsden, 1938) and similarly Lamoreux (1940) reported 60·9 per cent. fertility in eleven artificially inseminated fowl hens which with natural mating had only a 2·5 per cent. fertility.

In all the work on artificial insemination available, the route of entry for the dose of semen given was consistently confined to the female external genital orifice and the adjoining portions of the tract viz.; vagina, cervix, uterus.

(2) *The time of entry.*—Artifiical insemination has eliminated the necessity of bringing the male and the female together and waiting for coitus to take place; but in order to be successful it is bound by the natural limits of the time that fertilizable ova are available in the female. Attempts were made at the artificial induction of ovulation by hormone treatment (Quinlan and van der Wath, 1943). As a rule, however, artificial insemination has been carried out during the " fertile period " or at the time of optimum fertility which has been worked out for domestic and laboratory animals in relation to the onset and end of heat (oestrus) (Quinlan e.a. 1936; Kusnetsowa, 1932; Beshlebnov, 1938; Hammond, 1940; Quinlan e.a. 1941).

In the fowl Moore and Byerly (1942) found that artificial insemination was followed by a better fertility if carried out during the time when the oviduct contained an ovum being formed into an egg. Following insemination with no egg in the oviduct 51 per cent. of cases were fertile, whereas with the egg in the uterus

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only 39 per cent. of cases were fertile. Malmström (1943), Warren and Gish (1943) and Gracewski and Scott (1943) when submitting results in support of this finding showed that for general purposes, 2.00 p.m. to 3.00 p.m. was the best time of the day for inseminating fowls in view of the relation between oviposition and daylight, i.e. most eggs are laid in the forenoon (Atwood, 1929). Owen (1941) found that the best time for successful fertile insemination of pigeons was from one to eight days before the laying of a clutch.

THIRD STAGE.—VIABILITY AND MIGRATION OF SPERMATOZOA AND OVA IN THE BODY OF THE FEMALE.

A. *The Spermatozoa.*

In review of the data on sperm physiology, Phillips (1935) stated that spermatozoa may traverse the genital tract between the site of deposition and the site of impregnation, by the following agencies:—

(i) The force of the ejaculation.

(ii) The piston-like action of the penis.

(iii) Suction of the uterus.

(iv) Capillary attraction of the cervical canal.

(v) Ciliary action.

(vi) Muscular movement.

(vii) Pressure of the Viscera.

(viii) Motility.

Parker (1931) supplied evidence in a list of recorded times for spermatozoa to reach the upper end of the tubes after coitus e.g.—

Rabbit: 2·75 hours (Henson, 1876; Walton *e.a.* 1928).

Rat: 2 minutes (Hartmann and Ball, 1930).

Guinea-pig: 15 minutes (Leukaert, 1853).

Dog: 18 minutes.

Sheep: 5-6 hours (Quinlan *e.a.* 1932) 20 minutes (Scott and Phillips, 1941).

Pig: 5 hours.

[Starke (1945) has since demonstrated spermatozoa in the tubes of the ewe six (6) minutes after coitus].

Hammond (1940) maintained that sperm at the upper end of the tubes did not all arrive simultaneously and that they degenerated soon after arrival. Therefore as they reached this locality over a period after successful coitus, a supply of sperm was always present to fertilize any ova available during a specific period after each coitus. The length of this period has been determined for some animals e.g.—

Rat: 17 hours.

Rabbit: 24-30 hours (Hammond and Asdell, 1926).

Guinea-pig: 41 hours and

Sheep: 48 hours (Quinlan *e.a.,* 1932); 24 hours (Green and Winters, 1935) and $28\frac{1}{2}$ hours (Anderson, 1941 c.).

Crew (1926) considered that the fowl sperm reached the ovary in 24 hours after coitus. Hammond (1940) thought 2-3 hours was sufficient. Mimura obtained results after artificial insemination in which live sperm, dead sperm and carbon particles injected into the uterus reached the ovary in 26 minutes. The upward journey was found to be favoured if no egg was being formed in the oviduct (Gracewski and Scott, 1943) and by the pro-ovarian ciliary band which Parker (1931) demonstrated along the dorsal oviduct mucosa and which reaches from the uterus to the infundibulum. Retroperistalsis probably also plays a part (Parker, 1931; Curtis, 1916; Mimura, 1939). This journey of sperms is retarded by active secretion and egg formation (Parker, 1931; Mimura, 1939; Malmström, 1943; Moore and Byerly, 1942).

Spermatozoa were seen in the oviduct by Payne (1914) after killing hens at various intervals following on separation from the cock. He described a much reduced size for sperm after 14 days but found no change in motility up to the 56th day. Anderson (1922) recorded motility of spermatozoa in the oviduct at the latest after 15 hours and observed that sperm life in the hen's oviduct is not longer than in the mammalian female tract. Warren and Kilpatrick (1929) working with laying hens and using only a particularly virile Barred Rock cock, examined mucosal scrapings from infundibulum and from the uterus, and demonstrated spermatozoa at $6 \cdot 17$ hours and 24 hours; and also on the 12th, 15th and 18th day following copulation. At the sixth hour a large number had already lost the flagellum. Both at the sixth and 17th hours, those retaining the flagellum showed this organ considerably shortened. At the 24th hour a flagellum was rarely found. After this stage there were no visible changes in the sperm except that they became shorter and more crescent-shaped. They state that since the flagellum functions as a propelling structure, its loss soon after the spermatozoon had progressed up into the infundibulum may be the explanation of the advantages observed in fresh sperm over stale ones. Since sperm are capable of fertilization after the loss of the flagellum, it would seem that this is not their only means of locomotion. The worm-like contortions of some sperm-heads shown in the prepared material, may be taken to indicate that this part of the sperm is sufficiently flexible to allow motion. Although the flagellum may not be necessary for locomotion, it probably facilitates it, so that the sperm lacking this structure are not able to compete with those possessing tails. Munro (1938) found that at body temperature sperm in albumen and infundibular secretions became immotile but that they remained motile at these temperatures in uterine secretion.

Walton and Wetham (1933) made an unsuccessful search of the oviducts of killed hens for spermatozoa in the period from one to fifteen days after separation from the cock. In spite of extensive examinations both of the contents of the peritoneal cavity round the ovary and of smears taken from different parts of the oviduct no sperm could be found, although the eggs were perfectly fertile.

The lack of data on the position of sperm in the hen is evident from the contrasting opinions found in text books (e.g. Vermeulen. 1929; Hammond, 1940); Vermeulen (1929) maintains that spermatozoa await ripening ova outside the oviduct in the body of the hen for periods up to three weeks. He refers to the experiments of Ivanov (1924) who injected weak solutions of iodine into the oviducts of hens after mating and removal of the cock and yet collected fertile eggs from such hens for several days afterwards. Ivanov believed the sperms to enter and fertilize unripe ova. Although his findings were confirmed by Walton and Wetham (1933) with even more reliable spermicides, these workers could not subscribe to the view that sperm cells penetrated the follicular wall, but they were also by no means convinced that the opinion of Payne (1914) and Warren and

Kilpatrick (1929) viz. that tailless spermheads could impregnate ova, was an acceptable theory. Hammond (1940) discounted the views of Ivanov (1924) and Vermeulen (1929) on the grounds that embryonal development of all eggs when laid was at the same stage and they must therefore have been fertilized at an equal period before laying. It is significant that Warren and Kilpatrick (1929) found no difference in morphology or in relative abundance of sperm heads between the upper end of the oviduct and the region near the vent, even on the 18th day after separation.

Parker (1931) mentioned that active spermatozoa had been found in large numbers around the ovaries of birds and reptiles and Dulzetto (1937) described storage of sperm in cup-shaped depressions in the follicles of *Gambusia holbrooki* which findings bring to mind the spermatotheca that exist in many invertebrates.

Most writers refer to the life duration of spermatozoa in the body of the hen, in terms of the period during which fertile eggs were laid after separation of the cocks and the hens. Nalbandov and Card (1945) mentioned the " well established fact ", that avian sperm unlike mammalian sperm, are capable of surviving over a long period of time in the reproductive tract of the female. The environment in the hen's oviduct appeared to them as congenial for the spermatozoa as that in the vasa deferentia of the capon. There sperms had been shown to survive for as long a period following castration, as they do in the oviduct of the hen separated after mating. According to Crew (1926) the indications were that sperms could survive as long as 23 days in the body of the hen, but even he obtained a fertile egg 32 days after separation.

Fertile eggs were collected from fowl hens up to the following limits after the last copulation.

5th week: Barfurth (1896).

12th day: Waite (1911).

20th day: Waite (1911).

16th day: Payne (1914).

18th day: Chappellier (1914).

14th day: Ralf (1916).

15th day: Moore (1916).

19th day: Elford (1916).

15th day: Phillips (1918).

17th day: Laurie (1919).

12th day: Kauff (1919).

19th day: Fronda (1926).

32nd day: Crew (1926).

21st day: Curtis and Lambert (1929).

19th day: Chlebaroff (1930).

29th day: Nicolaides (1934).

25th day: Parker, McKenzie and Kempster (1942).

34th day: Nalbandov and Card (1943).

18th day: Raimo (1943).

The number of fertile eggs obtained, varied from six (Chappellier, 1914) and eleven (Curtis and Lambert, 1929) to fourteen (Nicolaides, 1934) and eleven (Raimo, 1943).

The following factors have been shown to influence the fate of spermatozoa in the female tract: —

(i) Species (Yochem, 1929; Quinlan *e.a.* 1941).

(ii) Stage in oestral cycle (Kugota, 1929; Quinlan *e.a.,* 1932; Quinlan, 1932).

(iii) The Chemical and Physical conditions (Ivanov, 1913; Crew, 1926; Quinlan *e.a.,* 1932, 1941, 1936).

(iv) The leucocyte contents (Walton, Hammond and Asdell, 1926).

(v) The amount and quality of semen (Walton, 1927, 1933; Parker, 1931; Phillips, 1935; Hammond, 1940; Milovanov, 1940).

(vi) The locality of discharge (Munro, 1938; Kusnetsowa *e.a.,* 1932; Milovanov, 1940)

In most animals, the keeping qualities of spermatozoa appear to be reduced much more by the secretions of the female genital tract than by those of the male (Hausman ,1879; Hühner, 1913; Giles, 1919; Hutchenreiter, 1915; Webster, 1921; Hammond and Asdell, 1926; Yochem, 1927; Quinlan, Marè and Roux, 1932, Kusnetsowa *e.a.,* 1932). The peculiar retention of sperms in the female bat for periods up to 90 days (Redenz, 1926, 1929; Folk, 1940) is not an exception, as in the epididymis of the male a life duration of seven months was recorded (Nakano, 1926). The sperms have been found harboured in the tubes, uterus and vagina of the hibernating female (Hartman, 1933; Mathews, 1937). Kugota (1929) found the uterine secretion of the rat to influence the length of the period of survival of spermatozoa more favourably during oestrus than in the interoestral period. Yochem (1929) found no difference between the effects of female secretions on sperm-life in the guinea-pig during the different times of the oestrous cycle, and neither did Quinlan *e.a.* (1941) in the sheep.

Quinlan *e.a.*(1932) discovered that spermatozoa in the genitalia of the merino ewe retained their viability for different periods in different sections of the genital tract, the most favourable being the cervix, which functions as a reservoir, from which the spermatozoa continue for a period to travel towards the upper tubes for the purpose of impregnation. Rabbit sperms were mostly agglutinated in the vagina and uterus and only a few reached the upper end of the tubes after each coitus according to Phillips (1935).

In the case of the domestic fowl, very little information on the effect of genital secretions on the life-duration of the spermatozoa is available. Adamstone and Card (1934) mentioned that it was commonly held that the secretions of the oviduct supplied nutriment to the sperms but there was little or no proof to support such a view. A deleterious effect on sperms had been shown to exist in other animals. The secretions of the fowl were studied by Cushny (1902), Surface (1912), Pearl and Curtis (1912), Curtis (1915, 1916), Bradley (1928), Buckner and Martin (1929), Conrad and Phillips (1938), Scott and Huang (1941) and McNally (1942), and are mainly concerned with the function of egg-formation. Since egg-formation in the hen is a continuous process over long periods (Asmundsen, 1931; Atwood, 1929; Warren and Conrad, 1939); and as ovulation is normally followed by a vigorous secretion of albumen (Pearl and Curtis, 1912; Richardson, 1935; Asmundsen and Burmester, 1936; McNally, 1942), the activity of the mucosa must have great influence on the fate of spermatozoa. This was shown to be the case by Mimura (1939), Malmström (1943) and Moore and Byerly (1942) who demonstrated the difference in chances of fertilization of eggs from semen introduced into the vagina at different stages in the formation of the egg in the oviducts

of fowls. Some results have been mentioned in connection with the time of insemination but further data were presented by Malmström (1943) when he showed that following artificial insemination with 0·05 c.c. fresh semen a 70 per cent. fertility was obtained if a membranous egg happened to be in the uterus at the time; but only 32 per cent. fertility resulted if insemination was done when a hard-shelled egg was in the uterus.

NB.—*Notes on the relation between egg-production, condition of the oviduct and fertility (as indication of sperm survival) in the fowl.*—In the fowl hen the ripening of ova takes place in clusters or clutches, each cluster producing a sequence of eggs, of one per day (Warren and Conrad, 1939; Atwood, 1929). According to Warren and Scott (1938) the time of ovulation is regulated by the sequence of maturation of the ova, rather than by the expulsion of the previous egg. As a result, secretion in the oviduct is periodical, almost daily, and begins at the infundibulum, increasing successively in weight of protein deposited around the yolk per unit of length of oviduct traversed as it passes down to the uterus (Pearl and Curtis, 1912). Secretion in the albumen region is markedly stimulated at any given spot on the mucous membrane just before the developing egg passes, and it continues for some time afterwards, the albumen being conveyed down to the egg even after it has reached the uterus (Richardson, 1935). According to Lamoreux (1940) larger and more frequent clutches favoured the fertility of the eggs laid by hens mated continuously in fowl-runs. This he explained by the greater sexual receptivity of the hens with the higher intensity of egg-production and he based his hypothesis on a test carried out with 65 hens. He cited Heuser (1916) who reported an average of two copulations in 13 days for hens which laid on less than half of the days and 21·8 for hens which laid on more than half the 13 days of observation. Wilkins (1915) reported similar results.

Warren and Kilpatrick (1929) found that the period between the removal of the male and the end of fertility was not influenced by the rate of production of the fowl hen. Their result was examined with the purpose of showing that high egg production did not carry off the available sperm supply faster. It can also be seen in their data, that the hens producing less than an egg on 59 per cent. of the days had a shorter duration of fertility, viz. 10·40 days, than hens producing over 60 per cent. which had an average duration of fertility lasting 12·04 days. Nicolaides (1934) found a small amount of evidence that fertility tends to be higher in the more intense layers. Those of his birds which produced more than the mean gave 7 per cent. better fertility than those that produced less than the mean. Funk (1939) showed that the eggs laid in clutches of several eggs are more fertile and possess higher hatchability than eggs laid in clutches of a single egg each. This is the case whether the different types of clutches are laid by the same hen or by different hens. Lamoreux (1940) demonstrated that conditioning of the oviduct for egg-production, which can be brought about by oestrogen injections in non-laying pullets, and which has a seasonal rhythm in adult hens, is not a factor increasing either sexual receptivity or duration of fertility from artificial insemination. Yet oestrogens are known to be produced by the larger follicles (Marlow and Richert, 1940; Allen and Whitsett, 1924) and it is also known that ovarectomy leads to atrophy of the duct similar to that which occurs when a hen ceases to ovulate.

In the second experiment of Nalbandov and Card (1943) where egg-production was on the wane, decreasing from 213 eggs in the first 5-day period after separation of the males to 71 eggs in the period from the 25th to the 30th day, fertility dropped to less than 2 per cent. after the 20th day. Whereas with the hens that maintained consistent production (table one of their publication)

fertility was still 10 per cent. after the 20th day and actually showed a slight increase in the period between the 20th and 25th day. Thus here a higher rate in decline of fertility was associated with a fall in egg production.

The relation between ovarian activity and sperm survival in the hen is thus real and actual but it is not clear just how the ripening of the ova affects the storage of spermatozoa. Walton and Wetham (1933) in submitting experimental evidence conforming with this fact, point out that the number of fertile eggs obtained from a hen after separation from the male, may exceed the number of ova of large size present in the ovary at the time of separation. The conditioning of the oviduct must be looked upon as a parallel occurrence to the length of time fertile eggs may be obtained as it is the result of the same ovarian activity (Allen and Whitsett, 1924; Lamoreux, 1840; Marlow and Richert, 1940; Munro and Kostin, 1943).

Crew (1926) found, however, no striking differences between the duration of fertility in hens that were active layers and in pullets that commenced laying 11 to 23 days after copulation; but Munro (1928) stated that if after one insemination a hen laid several fertile eggs and then five infertile eggs in succession thereafter, it was very rare for her to lay any fertile eggs until reinseminated.

B. *The ova (existence and migration).*

The life of the ovum on its journey from the ruptured follicle down the genital tract is very short in most mammals and in birds, unless it develops into a new individual. Rabbit ova have been observed through the Katch-Borcher's window and detailed descriptions of the life of the cells in this species have been published (Pincus, 1930; Hammond, 1934; 1940; and others): Many ova are liberated at the same time about ten hours after coitus in this animal. Ovulation is not explosive and as the cells emerge, the follicular fluid clots around them. From four to six hours later they separate out of this clot and are then open to fertilization until they begin to move down the fallopian tube where a thin albumen coat is deposited on their surface membrane. The cells are of course non-motile, and muscular and ciliary action is responsible for transportation. After ten hours all ova are usually coated with albumen and have begun to pass down the tube. On the third to the fifth day the ova reach the uterine mucosa at the apex of the uterine horn and if not fertilized degeneration has set in. Parthenogenesis is possible *in vitro*, where the morula stage may be reached but *in vivo* only degenerative fragmentation occurs, in which the nucleus takes no part that can be observed. Fertilized ova undergo segmentation and development in the tubes (Pincus 1930, 1936; Hammond, 1940). With variations the same processes occur in other animals, but Evans and Cole (1931) obtained evidence to show that the ovum of the dog may be fertilized for at least four (4) days after it had been shed. Blandau and Young (1939) studied the life of the Guinea-pig ovum and Blandau and Jordan (1941) the life of the Rat ovum. In the absence of the clotted follicular fluids the first few hours of life are different from those of the Rabbit ovum. The Ferret was found to ovulate 30 hours after coitus (Hammond and Walton, 1934a). By slaughtering ewes, Quinlan *et. al.* (1931) showed that in Merino ewes ovulation occurs about six hours before the end of œstrus, or roughly 36 hours after the commencement of an average heat period. The short fertilizable life of the merino ovum is indicated by the lowered fertility of ewes following service later than the 30th hour of œstrus (Quinlan *e.a.* 1932).

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The ferret ovary is enclosed in an ovarian capsule formed by the pavilion (Hammond and Walton, 1934). In the sheep, Quinlan and Mare (1931) observed the very close investment of the ovary at ovulation by the ovarian pocket and the fimbriated extremity of the fallopian tube intimately applied to the Graaffian follicle near the point of rupture. The peculiar relative restriction of the ovulation fossa in the mature ovary of the mare is interesting in this connection. Migration of ova into the abdominal cavity is thus exceptional and abnormal. Muscular action assisted by the anatomical position and possibly somewhat by gravity maintains ova in the pavilion of the tube and gradually force them towards the anterior entrance of the oviduct (Kiwish, 1851; Lode, 1894; Gerhardt 1905; Westman, 1929). Ciliated epithelium assists muscular action and in mammals ciliary activity is uniformly outwards. Westman (1929) showed that sexual activity in the rabbit. and monkey greatly increased the vigour of muscular activity of the fimbriæ and walls of the tubes.

Ovulation in the fowl was studied by Coste (1847), Warren and Scott (1935a, 1935b, 1936) and Phillips and Warren (1937). Normally only one ovum is liberated at a time and if it occurs on a day on which an egg is laid, it happens fourteen to seventy-five minutes after oviposition. The " yolk " is liberated by rupture of the follicle wall at the " stigma ", and rupture is instantaneous but the stimulus is not fully understood. Proof is available that it is not due to muscular action. nor to a last minute deposition of the yolk, nor to enzyme action, nor to changes in blood pressure and finally not to vacuolization of the stigma cells. No liquor folliculi was observed and the yolk mass at liberation was found to be so loosely enclosed in the vitelline membrane that the ovum assumed the shape of the cavity in which it fell. The grasping of the follicle by the infundibulum was entirely at random and not a causative factor in ovulation. After the infundibulum came in contact with the released ovum the enclosure of the ovum was found to be almost wave-like, with repeated advances and recessions of the edge of the infundibulum over the surface of the ovum. The average time occupied by this act was thirteen minutes but a maximum of twenty-fixe minutes was recorded. When once the ovum was enclosed entirely, the heavier muscles came into play and the process was more rapid. The pressure exerted by the muscles in the cephalic end of the magnum was very evident by the distortions brought about in the shape of the ovum. The yolk mass became much elongated and at times constricted in the centre by the wave-like contractions of the muscular wall of the duct. As soon as the ovum was entirely enclosed by the infundibulum it appeared to act as a stimulus to the whole oviduct. Peristaltic activity throughout the oviduct, including that portion through which the egg had already passed, seemed to continue during the period the egg was traversing the length of the organ. Cushny (1901) studying the hen's oviduct and Harper (1904) working with the pigeon, each state that after the egg leaves the infundibulum it is carried through the oviduct entirely by ciliary action, but Warren and Scott (1935) believed peristalsis to play a part, the muscular contractions being obscured in the region, where the egg happened to be, by the local distension of the oviduct. Parker (1931) referred to peristatic and anti-peristatic contractions that could be observed in the oviduct and he also demonstrated a pro-ovarian band of cilia along the dorsal quarter of the avian oviducal mucosa extending from the uterus to the infundibulum.

The hard-shelled egg which rotates obliquely during shell-formation in the uterus, probably often turns about, as in the fowl hen 10 per cent. to 30 per cent. of eggs are laid large end first (Olson and Byerly, 1932) but it is formed in

the oviduct with the large end anterior (towards the infundibulum) e.g. when the shell-membrane is produced in the isthmus. Oviposition is a solely muscular action in birds, occurring 20-30 hours after ovulation in the fowl and 39-44 hours after ovulation in the pigeon.

FOURTH STAGE.—IMPREGNATION OF OVA AND FUSION OF MALE AND FEMALE NUCLEI.

When an infertile laying hen is placed with the male, eggs which are present in the oviduct are not fertilized. Presumably, therefore, fertilization occurs not later than the entry into the top end of the oviduct. Harper (1904) found ova fertilized before they had entered the oviduct and suggested that the sperm might penetrate the follicular membrane of the ripe ovum when the membrane was very thin so that the egg was fertilized before being released. This assumes the constant presence of fertile sperm in the neighbourhood of the ovary and according to Walton and Wetham (1933) such sperm could not be found. In order to account for the failure to find spermatozoa in this situation, Ivanov (1924) postulated that almost immediately after insemination the sperm penetrated not only the follicular membranes of the ripe ova, but also those of the immature, and in this way a whole clutch of eggs ripening on the ovary might be fertilized at once. Crew (1926) found that by the introduction of a second male amongst fowl hens the period of duration of fertility from the first cock was reduced by about 50 per cent. Walton and Wetham (1933) state that it is therefore clear, that if Ivanov's hypothesis is correct, replacement of spermatozoa within the immature ova must take place. As has been mentioned this hypothesis was discarded by Hammond (1940) on the grounds that all eggs have reached the same stage of development at the time of laying.

In the pigeon and fowl the spermatozoon enters the ovum apparently with the acrosome first. Actual penetration of the vitelline membrane is most likely to occur immediately after ovulation as no follicular fluids hinder the sperm at this moment and McNally (1942) showed that on contact with the infundibular mucosa the vitelline membrane was swollen and strengthened by its secretions, (see van Drimmelen, 1945b). Further evidence from the work of Harper (1904) and Patterson (1910) had recorded the occurrence of polyspermy in the fowl (5-25 sperm heads) and in the pigeon $\pm$ 6 sperm heads (M. Blount, 1907).

Hammond (1940) remarked that not until large numbers of sperms have accumulated at the tops of the oviduct as a result of several matings, are all the eggs laid, fertilized, and maximum fertility was not attained until about the sixth day after placing hen and cock together. This view is perfectly supported by the results of about 15 single inseminations performed by Moore and Byerly (1942) involving the incubation of 1,046 eggs.

Parker (1931) states that active sperms have been found abundantly about the ovaries of both birds and reptiles and he relates the case of the turtle which in winter had its ovary covered with fluid full of active sperm although no ovarian eggs had been freed. The cup-shaped depressions on the follicles of *Gambusia holbrooki*, a turtle, described as sperm-reservoirs by Dulzetto (1937) permit entry for the sperm through an opening when ripe and the fertilized ova commence development within the follicle.

A "sperm swarm" was observed in mammals. (Hammond and Asdell, 1926; Hammond, 1940, Walton, 1938b). This term implies that the site of impregnation is reached by the first spermatozoon of a single insemination at a more or less regular period of time after discharge at the lower end of the

tract. It is followed by greater and greater numbers until a peak in numbers available at the site is reached, not by accumulation, as the earlier arrivals apparently lose their fertilizing powers fairly soon (Quinlan *et al.*, 1932), but by greater numbers of cells attaining the locality simultaneously. After the peak moment the numbers wane for a time till the last fertile cell has lost its functional ability again at a more or less determined moment in relation to the time of insemination.

Thus in the rabbit which ovulates 10 hours after coitus, sperm naturally reach the ovary seven hours before, and remain available until about 23 hours after ovulation. But if insemination is artificially caused to take place less than 2 hours before ovulation or more than 28 hours before, then smaller litters of young are obtained. Thus the female can only be successfully inseminated before the end of the period of fertilizable survival of the ovum, less the shortest time of migration of sperms over the length of the tract, and prior to this moment for the period sperms retain their fertilizing power, (Hammond and Asdell, 1926; Hammond, 1934; Hammond and Walton, 1934).

On this basis, in respect of fertility alone, in the fowl, an ovum may be impregnated from an insemination 32 to 35 days before ovulation (Crew, 1926; Nalbandov and Card, 1943) right up to 26 minutes before the ovulation (Mimura, 1939).

Dunn (1927) obtained a fertile egg from a hen 21 hours after a single mating; Martin and Anderson (1918) 29 hours, Curtis and Lambert (1929) 24 hours and Nicolaides (1934) $19 \cdot 5$ hours after.

Warren and Kilpatrick (1929) found sperms of a new male to supersede those of a previous male with continuous natural mating, on the third day after exchange. They suggested that the difference between their results and those of Dunn (1927) may have been due to the fact that the latter was working with virgin females whereas their hens were already fertilized. Considering it possible that impregnation might take place at a later stage in the egg's passage through the oviduct, Warren and Kilpatrick thought the position of older sperms at the top of the duct more favourable than that of sperms still passing up the duct. If the only sperm in the oviduct were those part of the way up, then the period between coitus and laying of a fertile egg would be reduced to a minimum.

Nicolaides (1934) who obtained a fertile egg $19 \cdot 5$ hours after mating birds singly in a cage, suggested that fertilization could comprise two distinct processes : —

(1) Penetration of the wall of the ovum by the sperm.

(2) Fusion of the nuclei of male and female gametes.

This worker referred to evidence from Harper's (1904) and Patterson's (1910) observations that the two processes do not occur in the same locality nor at the same time.

The activity of spermatozoa apparently play a role in detaching the ova of the Rabbit from the follicular clot (Hammond, 1940; Pincus, 1930). The role of hyaluronidase was discussed in this connection by McClean and Rowlands (1942) who mention that the action of this enzyme, viz.: liquefaction of the gel suggests an explanation why such enormous numbers of sperms are produced for fertilization. Large numbers may be required to produce the necessary concentration of hyaluronidase to liquefy the hyaluronic acid gel and

to allow penetration of the egg by the single effective sperm. This view was supported by results of Fekete and Duran-Reynols (1943) who found Hyaluroni-dase very active in dispersing follicular cells surrounding the ova of mammals. Pincus (1930) observed polyspermy in rabbit ova.

Selective fertilization was studied by Milovanov (1941), who inseminated recessive coloured rabbit does with mixed semen from dominant and recessive bucks. From small doses 49 young were obtained and all were cross-bred; 20 per cent. were weaklings and only two out of every seven were males From large doses only 8 per cent. were weaklings, about half were cross-bred and about half were males. Thus the result was probably not due to selective fertilization but the reduction of the dose may have influenced the quality of the sperm and the vitality of the progeny.

Dunn (1927), Curtis and Lambert (1929) and Bonnier and Trullson (1939) reported inconclusive results with experiments in selective fertilization in fowls, but Parker, McKenzie and Kempster (1942) considered that fertilization of the ova by competing sperm from different cocks was largely a matter of chance, although they admit that compatibility of sperm and ova might be a factor to consider and that further work in this field would be desirable. These workers obtained conspicuously fewer chicks from White Leghorn semen when this was mixed with New Hamshire and Barred Rock semen and inseminated into New Hamshire females.

FIFTH STAGE.—MIGRATION AND DEVELOPMENT OF THE ZYGOTE, EMBRYO AND NEW-BORN YOUNG.

The qualitative influence of aged spermatozoa on the resulting zygote in the fowl was first exposed by Barfurth (1896) and this was confirmed by Crew (1926) and by Nalbandov and Card (1943). Soderwall and Young (1940) stated about sex cells generally, that both ova and sperm were short-lived cells, but whereas in the case of the ovum functional impairment occurred very soon after ovulation, no such subnormal period seemed to occur in the case of the spermatozoon. As a result of the functional impairment in ova, aged before fertilization, development suffered serious consequences. The ovum was studied in the rat and the guinea-pig from this aspect. Blandau and Young (1939) and Blandau and Jordan (1941) demonstrated the increased tendency of ova after delayed fertilization, to produce weak zygotes, which became resorbed, or were aborted or still-born. Hammond and Asdell (1926), working on the rabbit, submitted proof that the time of insemination could affect the litter size and birth weight of young in rabbits (Hammond, 1934; Hammond 1940). Apparently the deposit of albumen added on the surface of rabbit ova in the tubes renders them impervious to sperm when aged.

As regards the male cells Soderwall and Young (1940) decided that when dealing with spermatozoa the situation was always complicated by the competition between several sperm; the question could only be settled by development of a technique whereby fertilization could be accomplished by means of an isolated single spermatozoon.

Nalbandov and Card (1943) noticed that with increasing age of the sperma-tozoa of the fowl when stored in the body of the hen after removal of the cocks (" staleness " of sperm) a progressive decrease in the age reached by the embryos in eggs fertilized by them, was observed, i.e. the eggs laid later after removal of the male tended to develop less far, than the earlier eggs, on incubation. Graph B has been constructed from their data.

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In five weeks following the removal of their males, all the eggs laid by five groups of hens were set and 941 proved to be fertile. Hatchability remained above 50 per cent. in all groups during the first ten days but only in two groups during the period from the eleventh to the fifteenth day and only in one group from the 16th to the 20th day. After that all eggs laid failed to hatch though a considerable number were still found to be fertile. Counting the day of removal of the cocks as day number one, the last egg to hatch was laid on day number twenty i.e. on the nineteenth (19th) day after removal of the male.

GRAPH B.—The average age of the embryo at death in fertile fowl eggs laid in successive five-day periods following separation of the cocks from the hens.

(*Data from Nalbandov and Card*, 1943).
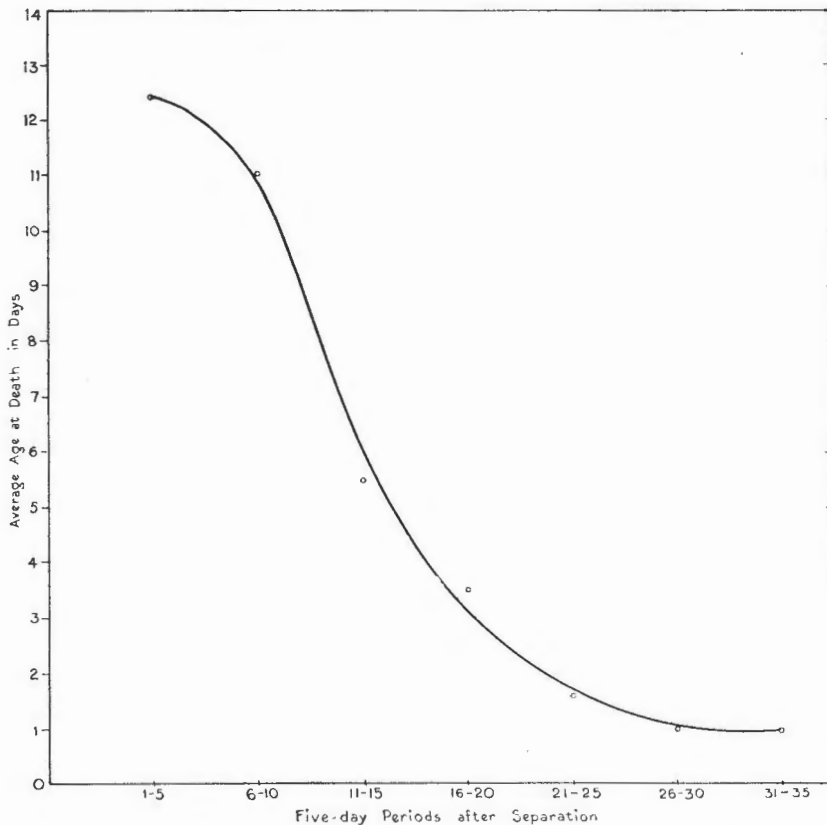
Graph B shows the incubation results of fertile eggs collected in each of seven, five-day time periods in fowl runs from which the cocks had been removed on the first days of the first five-day periods. The results are given in terms of the average age reached by the embryos in each of the five-day periods.

This finding supported the conclusion reached by Crew (1926) that moderately " stale " sperm could activate an ovum but the resulting zygote would fail to complete its development. Warren and Kilpatrick (1929) reviewed evidence that no unusual embryo mortality resulted from fertilization of eggs with sperm aged in the oviduct for not more than fourteen days, and this was supported by data of Parker *et al.* (1942).

Histologically such defectively fertilized eggs have been found to be abnormal in their cleavage processes, even though cleavage never progressed very far (Lau, 1894).

At this stage it should be noted that Hammond (1940) discredited this difference between the limits of fertility and hatchability on the ground that no comparable phenomenon occurs in the rabbit. Also, sperm aged in the guinea-pig oviduct resulted in development and gestation although fertilizing power was decreased (Soderwall and Young, 1904). Nevertheless, Young (1931) working with spermatozoa after prolonged isolation in the epididymis of the Guinea-pig, found a higher frequency of abortion when the older sperms were used, and suggested that such abnormal pregnancies might be attributed to the condition of the spermatozoon.

Munro (1938) discussed the question at length. He found that sperm, which survived unfavourable treatment e.g. dilution *in vitro,* did not manifest their experience by any vital effect on the embryo developed from the ovum they subsequently fertilized. The fact that general poultry experience finds high embryo mortality associated with the low fertility cannot be explained by a lack of normal vitality in the smaller proportion of functional sperm. If this lack were considered the cause of a higher death rate in the resultant embryos, then the existence of varying protoplasmice states within the sperm would have been taken as an accepted fact. That these varying states in the sperm could affect embryonic development, Munro considered a somewhat novel concept, which was not necessary to explain the correlation between fertility and hatchability. Variations within the egg, influencing both inpregnation and development offered a more satisfactory explanation. Munro found no correlation between fertility and hatchability at the various levels of semen dilution, as all fertile eggs from the lower dilutions hatched. He concluded, however, that the physiological state of the male parent might be reflected in the hatchability of the eggs. It was not stated how he meant this, but he may have referred here to fertilization of the egg at sub-optimum time, -site or -method, directly as the result of physiological abnormalities or sub-normalities of the male, coitus or of the spermatozoa, themselves. Munro considered that when fertilization was accomplished, the history of the developing embryo was largely controlled by the chemical composition of the egg. It is apparent, that the effects of inherited genes on embryonal development were not included in this discussion.

Riddle and Behre (1921) recorded a high embryo mortality in eggs of ring doves laid after the seventh day following the last coitus permitted. They considered both factors, viz.: The possible deficiency or weakness of the ovum in eggs laid more than six days after copulation, and the staleness or ageing of the sperms responsible for the short embryonic life-term of the embryos.

The normal development of the zygote in the pigeon and fowl begins in the oviduct. Coste (1847) described how the follicular orientation of the ovum was maintained in the oviduct after ovulation and Harper (1904) reported that normal cleavage took place in regular planes relative to the chalazal axis of the egg; but no explanation of these observations could be found in the literature and more attention to the subject is indicated. The data from Patterson (1910) show the pre-oviposition development of the hen's egg clearly. (See Table 2.)

Patterson (1910) assumed that 22 hours elapsed between impregnation and ovi-position in the hen and 41 hours between these processes in the pigeon. He stated that fertilization took place immediately after ovulation and that the fowl's egg during passage from the upper oviduct to 12 inches from the infundibulum

developed through all stages from maturation to the first cleavage spindle. The accessory sperm nuclei, 5-20 in the fowl, led to rudimentary accessory cleavage in the fowl, not to complete accessory cleavage as in the pigeon (Blount, 1909). All the accessory nuclei were at the central part of the blastodisc and this type of cleavage was in radial planes at the periphery. The two-celled stage was reached at three hours old, and though the axis position was not considered a fundamental law of development, the long axis of the embryo was consistently at right angles to the chalazal axis. Up to the eight cell stage all cells were open to the periblast in the hen, whereas in the pigeon's egg (perhaps to prevent influence from the accessory cleavage) marginal cells became closed (Blount, 1909). Patterson and Blount agree, that the increase in cell layers is derived from horizontal cleavage between the segmentation cavity and the surface of the blastodisc. Bartelmez and Riddle (1924) describe a mechanical basis for the origin of subgerminal cavity, in the water absorption of the yolk.

TABLE 2.

Pre-oviposition Development of the Hen's Egg.

Line.	Time of laying previous Egg.	Time of collection of Specimen.	Estimated Age in Hours after Ovulation.	Position in Oviduct.	Stage of Development.
a	1.30 p.m.	4.00 p.m.	2·5 hours	11″ from funnel	Pre-cleavage.
b	8.30 p.m.	2.30 p.m.	3·0 hours	Anterior Isthmus	2 celled.
c	8.30 p.m.	2.30 p.m.	3·5 hours	In Isthmus	4 celled.
d	10.00 a.m.	8.00 p.m.	4·0 hours	In Isthmus	8 celled.
e	11.00 a.m.	6.00 p.m.	4·5 hours	In Isthmus	15 celled.
f	12.30 p.m.	8.15 p.m.	4·5 hours	In Isthmus	32 celled.
g	3.45 a.m.	4.45 p.m.	5·5 hours	In Uterus	64 cells ⎫ In
h	9.30 a.m.	7.30 p.m.	7.0 hours	In Uterus	154 cells ⎬ surface
i	3.00 a.m.	7.00 p.m.	8·0 hours	In Uterus	346 cells ⎭ view.

Parthenogenetic development continued only as far as the 10th-hour-after-fertilization stage (Harper, 1904).

If the laying of the fertile egg is delayed for 20 hours development was equal to that of 20 hours incubation. (Patterson 1910).

Incubation.

Incubation of the eggs is a process providing environmental factors in the development of the avian egg, which are additional to the factors inherent in the zygote and those present in the parents, such as apply in the case of mammals.

Hatching is said to be affected by internal and external factors (Romanoff, 1931; Hutt and Greenwood, 1929; van Manen, 1932; Hammond, 1940). Although death of the embryo may happen at any stage of incubation, there are two main critical periods in the life of the chick embryo. These are approximately at the fourth and 19th day of incubation. In artificially incubated eggs the effect of the external factors is responsible for a greater mortality than the internal factors from the 18th day onwards. (Hutt and Greenwood 1929; Romanoff, 1931; Landauer, 1937; Hammond, 1940).

It was thought possible that another critical period occurred prior to incubation e.g. before gastrulation is complete. Chilling at this stage, for instance eggs which were laid in winter, and eggs laid quickly after ovulation [as in the middle of the clutch (Atwood, 1929)], might develop abnormally (Hammond, 1940). Funk (1934) could not show a decrease in proportion of teratologies or improve hatchability by keeping eggs at 101° F. for 6 hours after laying. McNally and Byerly (1936) on the other hand showed that eggs with intervals of 28, 29 and 30 hours betwen the laying of eggs, showed poorer hatching quality than eggs with intervals of 24, 25, 26 and 27 hours. They omitted reference to the relation between fertility, greater intensity of egg-production and shorter intervals between oviposition.

The time and temperature of keeping eggs between laying and setting has consequences similar to those described for aged sperm. Romanoff (1931) demonstrated reduction in hatchability after storage giving figures for 14 days, 28 days and 32 days in which cases hatchability was 80 per cent., 30 per cent. and 10 per cent. respectively. Van Manen (1932) mentioned the detrimental effect of storage temperatures outside the range 50°-60° F. and many authors have reported that low temperatures reduce hatchability (Scott, 1933; Warren, 1934; Funk, 1934). Recent experiments have proved, however, that storage temperatures of 32° F. and 38° F. for seven days had no influence upon hatchability when compared with similar eggs stored at the usual recommended temperature of 50° F., if the eggs were laid by hens known for producing good hatching results (Phillips, 1945). An interesting observation was made by Heywang (1945) during May to July in Arizona at maximal atmospheric temperatures up to 109·0° F. when he showd that hatchability was not worse, but better in fact, if the eggs were left in the poultry run and only collected once per day, than when collected more often and placed in the cool (55° F.) storage room immediately after laying.

Parker (1945) reported an important observation when he described signicant increase in hatchability of the fertile eggs laid by hens when they had been artificially inseminated in the late afternoon. This increased hatchability was shown to be related to the fact that the larger percentage of hens have a soft or membranous egg in the lower region of the reproductive system late in the day. No explanation was advanced by this author although he put the question, whether shell formation in the oviduct could affect the sperms present in the oviduct in such a manner as to determine the fate of the embryos resulting from the union of those spermatozoa with ova.

The many factors on which successful artificial incubation depends cannot be reviewed here, but in connection with the present work it is necessary to note that detrimental effects can be produced by the following factors:—

    (i) Poor ventilation (room conditions).

    (ii) Lack of moisture (South African climate).

    (iii) Overheating (oil burning incubator).

    (iv) Chilling [do (iii)].

    (v) Failure to turn newly set eggs daily.

    (vi) Disturbing eggs during the last three days before hatching.

    (vii) Opening the incubator during hatching (chill, loss of humidity) (Van Manen, 1932; Romanoff, 1931; J. A. van der Merwe, 1942; Landauer, 1937, 1941).