

THIRD CHAPTER.

OBSERVATIONS AND DISCUSSION.

The data reported in the following pages are collected from studies on the sex-physiology of birds, which can conveniently be grouped in the following phases:—

- (a) Observations on the semen collection from male pigeons and on the breeding habits and artificial insemination of racing pigeons.
- (b) Observations on the laying habits of fowl hens in battery cages, the collection of semen from fowl cocks and the artificial insemination of fowls. Four series of inseminations were performed:—

The *first* series of 150 inseminations, including 117 intraperitoneal operations, 22 control inseminations *per vaginam* by artificial methods and eleven controls with natural mating, was done at Bloemfontein; and provided details of the intraperitoneal technique for artificial insemination of birds.

The *second* series of 108 inseminations was carried out at Glen College of Agriculture, but the results could not be used.

The *third* series was again done at Bloemfontein, in order to collect information on the qualities of fowl semen samples and on spermatozoa stored *in vitro* and also *in vivo* in the hen; and included 80 cases of insemination (62 intraperitoneal, sixteen *per vaginam* and two natural matings).

The *fourth* series, during which 61 inseminations (forty-one intraperitoneal, eleven *per vaginam* and nine natural) were carried out, was done at the Onderstepoort Veterinary Research Institute, with the special object of determining the effect of insemination by the new intraperitoneal technique, on the location of the spermatozoa stored *in vivo* in the fowl hen.

In drafting this report, it has been found necessary to consider the data in twelve parts. These parts are presented in the general sequence in which work was done, showing in each the development of findings of the previous work. Therefore the conditions or circumstances under which the work was carried out are given first: "A", followed by the experimental results: "B". Section B. embraces the twelve parts: First the collection of semen (part 1.), then the insemination experiments of the first series (parts 2. to 8.); and after these the examination of the semen (part 9.) and the competitive relations of spermatozoa (part 10.). The discovery of the site of sperm storage *in vivo* in the hen (part 11.) and the demonstration of male cells in the tissues of isolated, fertile fowl hens with the aid of the intraperitoneal technique (part 12.) is given last as shown in the following list:—

A *The conditions of work.*—Giving details to show that such work could be done without laboratory facilities.

B *Experimental Results.*—Giving details on the work done and the record of results obtained:— parts 1. to 12. viz.: The record of the work done:—

- (1) To collect semen of pigeon and fowl cocks singlehanded.
- (2) To see if eggs may be fertilized by intraperitoneal injection of semen.

- (3) To determine the effect of intraperitoneal insemination on the egg-production and health of the pigeon and fowl hens.
- (4) To discover details concerning the fertility resulting from intraperitoneal insemination in pigeons and fowls.
- (5) To determine the hatchability of eggs fertilized by semen injected into the abdominal cavity of pigeons and fowls.
- (6) To study the effect of some qualities of the subject on the results of intraperitoneal insemination in pigeons and fowls.
- (8) To see if the results of intraperitoneal insemination were influenced by the technique, equipment and materials used.
- (9) To examine the semen of pigeons and fowls used for insemination.
- (10) To study the biological relations between fowl spermatozoa of different origin.
- (11) To discover the site of storage of spermatozoa in the body of the fowl hen.
- (12) To see if intraperitoneal insemination has an effect on the position of spermatozoa stored in the organs of the fowl hen.

A. THE CONDITIONS OF WORK.

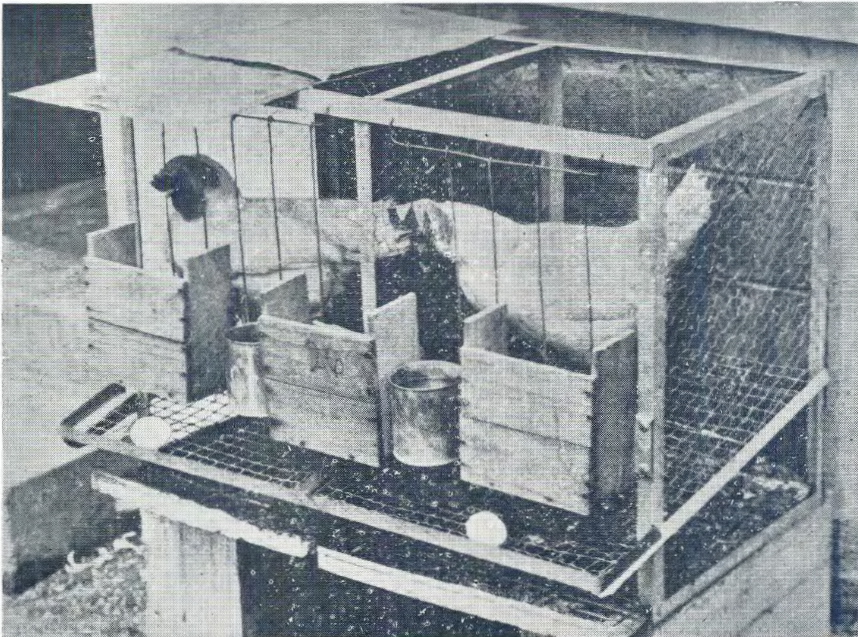
The work was essentially carried out under improvised conditions at a private residence in an urban area and except for the experiments conducted at Onderstepoort was not at any time included in the routine of veterinary duties. The result was that several observations had to be incomplete or valueless because of the absence of the investigator at critical times. For this reason the second series of fowl inseminations was arranged to be done at Glen College of Agriculture where a co-worker would always be in attendance. This attempt failed mainly through war conditions and through a shortage of staff; but although no results are available from this series, *the experience gained* was valuable. Generally all work was done in the morning or evening at 05:00 to 07:00 hours and at 17:00 to 24:00 hours, (Summertime September to April, one hour before South African time). It was impossible to obtain labour and assistance at these times, therefore modified methods had to be evolved for many procedures. The experiments were begun with racing pigeon hens, isolated in ordinary partitions in a loft. The birds were each paired up to a castrated cock with good results as regards sexual activity. Instead of a castrated cock, a salpingectomised hen was sometimes paired with a laying hen but later homosexually mated normal hens were preferred. These had to be selected in pairs of which the eggs of one partner differed markedly in size and shape from those of the other partner, a necessity occasioned by the fact that pigeon hens so mated, tend to develop coinciding sexual cycles, so that they both lay at the same time after the first few clutches have been produced (Owen, 1941). Most of these two-hen matings were confined in outdoor cages placed on top of fowl laying batteries, because the municipal regulations did not permit the keeping of pigeons and poultry in the garage. Some canaries and budgerigars were kept in the garage but no useful data were extracted from experiments with these birds.

Because the egg production of fowl hens is more prolific, these were used to obtain data quicker and in greater volume. Some hens which had been reared to observe the results of a colchicine alkaloid injection into the egg during the first hours of incubation, were available, as they had never been allowed to copulate and were several weeks in production. Other birds were obtained from local plants. The hens were placed in wood or metal home-made outdoor laying

ARTIFICIAL INSEMINATION OF BIRDS.

batteries, 45 cm. × 45 cm. × 45 cm. space per cage. They were fed on the commercial poultry laying mash registered under the name "Nasfeed" with water and oystershell grit *ad lib.*, and a handful of lucerne or barley greens cut in 1 cm. lengths and given at about 17·00 hours daily. Cocks and hens received the same ration. The mash hoppers were filled as a rule, but remained accidentally empty on less than 2 per cent. of the days and the greens were not provided on approximately 5 per cent. of the days owing to lack of help. In winter months 25 per cent. to 33 $\frac{1}{3}$ per cent. yellow maize meal was added to the mash. The laying battery accommodation was increased in stages from 6 to 14 cages, and four indoor batteries were at one stage provided in a post mortem room at the veterinary office, mainly on account of the effect of frost on the production of White Leghorn single comb hens and cocks. Some cases of frostbite occurred with a break in production immediately after. Provision of sunlight for the birds in the indoor batteries was somewhat irregular; the cages were placed outside in the sun on about one day out of every three from 08·00 hours to 13·00 hours. The outdoor cages had ample sunlight and in summer were shaded by a tree from about 11·00 hours till evening. All batteries faced due east so that direct exposure to the sun occurred during the morning on clear days. The cockerels were kept in the same cages as the hens. Some of the White Leghorn cocks had to be dubbed, not only to prevent frostbite but also because of the tendency of their comb and wattles to become so large as to interfere with feeding. [See figure (i)].

FIGURE (i).—The Laying-battery for Fowls.



Home-made battery cage for fowls, suitable for small numbers of birds under owner's care in urban areas. Each bird is kept in a partition measuring 45 cm. by 45 cm. by 45 cm., standing on wire screening, through which the droppings fall on to removable dropping boards. The screening slopes forward so that the eggs, when laid, roll slowly out of reach of the hen.

(Photo by "Farmers Weekly", November, 1944).

Semen collection was done in the loft in the case of pigeons, as the cocks were kept either in the stock lofts or in a separate partition in the racing loft. The latter had regular liberation for flying exercise. Fowl cocks were removed from the cages and taken into a garage for the purpose. Inseminations were performed indoors; eggs were collected once, twice or three times daily at irregular intervals. They were marked, weighed and recorded immediately and were set within eight hours after collection. The record of each egg specified the following items:—

- (1) Date laid or collected.
- (2) Weight at collection.
- (3) Description, i.e. normal, premature shelled, or premature membranous or cracked.
- (4) The time of oviposition i.e. time of inspection before and after laying of an egg.
- (5) The dates and results of candling.
- (6) The date of hatching and paternity of chick as determined by it's colour and comb when possible (see part 10).

At first eggs were incubated in a 60 egg "Hearson's Champion" still-air oil-burning incubator of modern type. As this machine was borrowed for a limited period only, it was later replaced by an older model of the same make that had been discarded years earlier as broken and useless, but could be repaired to a fairly satisfactory condition. An old 60 egg standing still-air "Buckeye" incubator with the hatching tray only, was brought into service later and all fertile eggs were, after the third to the fifth day of incubation, transferred to this machine for further development and hatching. Eggs were turned once and sometimes twice daily from the first to the last day. All incubation was done in a room rarely used for domestic purposes and candling was done by electric light, a bulb being fixed in a medium sized tin with an oval opening cut in the lid. Eggs were individually handled and tested three to five times if fertile, i.e.

First test: third to fifth day.

Second test: seventh to tenth day.

Third test: fourteenth to sixteenth day.

Fourth test: about the 18th day.

Fifth test: about the 22nd day.

The third and fourth tests were very often not carried out. Moisture was not sufficient although the watertrays were always full and hessian covers were used to increase evaporation. Ventilation may have been overdone but in view of the presence of eggs with advanced embryos in the machine, this was considered the lesser of the two evils.

Dead embryos, dead chicks, and their descriptions were recorded when the eggs were broken after each test. Hatchings were recovered without delay and for this purpose it was found necessary to mark fertile eggs on both ends, particularly the small end, for easy identification of the empty shell. The tray was divided in eight wire gauze partitions and only eggs of very different periods of incubation were placed together so that no more than one chick could appear in each partition over a period of forty-eight hours. Smears and materials for chemical and microscopical examination were transported two miles to the veterinary office for examination.

For the experiments at Onderstepoort (see part 12), a stable was obtained, and to reduce the lower extremes of temperature at night, electrical heaters were fitted on the floor under the incubators. Here semen collections, inseminations and incubation were all done in the stable, but the females were kept in an intensive poultry house on the plant, or in cages in a glass-walled isolation room.

It will be seen that whilst all experiments recorded here have been carried out under improvised conditions, essential records have been accurately made and every reasonable precaution has been taken to guard against avoidable errors in experiments and data.

B. EXPERIMENTAL RESULTS.

(1) *The Methods of obtaining semen from pigeons and fowls.*

(a) *Pigeons.*

No experience of semen collection from live birds had been gained before this work was started.

All semen samples previously handled had been extracted from the vasa deferentia of killed males. The method described by Owen (1941) was applied in the present work for reasons of economy, i.e. the semen was pressed from the bulbous ducts, while the bird was held in the left hand or in a paper funnel. The ejaculate was thus passively extruded and collected by means of a glass pipette with a rubber teat and graduated to measure 0.001 c.c. The collector had to hold the bird himself in the absence of any help. On account of struggling the right hand was required to control the bird, and the pipette was held in the mouth. This presented great difficulty due to lack of proper visibility and wastage in transferring the pipette to the hand. Struggling was such a common occurrence that this method resulted in the loss of the material. All the birds were racing homers and their temperamental disposition must be held responsible. In order to evolve a satisfactory method of collecting semen single-handed a new technique was required. Experiments done for this purpose are recorded below:—

EXPERIMENT (1).

Object: To develop a satisfactory technique for semen collection from excitable pigeons.

Method: Pigeon cocks were controlled by placing the birds, head downwards, between the knees of the seated operator. His left hand held the tail of the bird. The knees were held slightly apart and the thighs were horizontal. The back of the bird was facing towards the left, with the sternum towards the right side of the operator. His left hand held the tail of the bird at right angles to its back by pressing the palm of the hand against the ventral surface of the feathers. The left thumb and fore-finger were thus able to grasp the vent, in the gentle milking action, applying some pressure to the abdominal wall. [See Figure (ii)]. The instruments were handled by the right hand which was entirely free for the purpose. The pipette used at first was later replaced by applying the insemination syringe direct to the semen on the vent, to collect it by aspiration.

Results: The summary of semen collections performed on pigeons is given in table 3. The total number of Pigeon cocks used was 34, some being available throughout the four seasons of experimentation. Only three of these birds gave regularly more than 0.01 c.c. per collection in

their yearling season and only two, birds Nos. 136 and P.53, supplied that amount in their second year. The improvement of the success and of the volume obtained at collection was mainly due to the introduction of the new technique as is evident from the summary in table 4. In the fourth season, however, almost all birds used were old birds so that in both respects a drop occurred.

FIGURE (ii) (a).—The Collection of Pigeon Semen.



Position of operator for the collection of pigeon semen by the author's modification of the abdominal massage method of Burrows and Quinn (1935), and Owen (1941).
(Photo by Author).

TABLE 3.

The Semen Collections Performed on Pigeons.

Line.	Season.	Number of Cocks used.	Number of Trials.	Percentage of Trials Successful.	Average Amount of Semen obtained per Successful Trial.
a	First.....	16	288	17%	0·0042 c.c.
b	Second.....	29	485	72%	0·0046 c.c.
c	Third.....	28	305	87%	0·0053 c.c.
d	Fourth.....	17	85	85%	0·0035 c.c.
e	TOTAL.....	34	1,163	—	—

ARTIFICIAL INSEMINATION OF BIRDS.

FIGURE (ii) (b).—The Collection of Pigeon Semen.



The position of the fingers for the collection of semen from pigeons by the author's modification of the abdominal massage method of Burrows and Quinn (1935), and Owen (1941).

(Photo by Dr. A. T. Nesor, Bloemfontein).

TABLE 4.

The Results of Different Methods of Semen Collection in Pigeons.

Line.	Method.	Number of Cocks Used.	Number of Trials.	Percentage of Trials Successful.	Average Amount of Semen obtained per Successful Trial.
a	Bird held with two hands and pipette held in the mouth....	25	358	17%	0·0038 c.c.
b	Bird held by left hand on knees and pipette held in right hand	33	805	83%	0·0048 c.c.
c	TOTAL.....	34	1,163	—	—

The figures give the results on similar groups of birds, many being used in both groups and thus it is clearly shown that calm surroundings and proper control of pigeons are of importance especially in respect of the number of attempts likely to be successful.

The results were best in the spring months, and in the evening after dark. Usually the racing cocks were not in any way permitted contact with the hens but the stock birds were mated and were active breeders. The latter provided useful amounts of semen when sitting on eggs but not when "driving" the hens. Removal of birds from the loft to the garage disturbed their capacity for the release of semen to some extent. The best response was obtained in the loft, the birds being lifted off the perches and replaced immediately after collection. One bird could be tapped in this way two or three times per evening and might provide 0.045 c.c. of semen on one day. In the first and second seasons the average volume of semen was markedly increased as the result of the modified collection technique. In the third season the improvement was to some extent due to selection of suitable males and omission of poor producers.

(b) *Fowls.*

The methods used for obtaining semen from cocks were that of Parker (1939) and that of Burrows and Quinn (1935, 1937). With the former a glass receptacle 4.5 cm. diameter and 2.3 cm. deep was attached over the vent of a cock when he was permitted to "tread" a hen. It could be carried out without help, but was found laborious and time-consuming on account of the handling of birds and waiting necessitated. The latter method required the help of an assistant to hold the cock and this was an insurmountable drawback in the existing circumstances. In view of the success achieved in handling pigeons single-handed it was decided to develop a similar method for handling fowls without assistance. The procedure is given in the second experiment.

EXPERIMENT (2)

Object:—To develop a method of collecting semen from fowl cocks without requiring the help of an assistant.

Method:—The bird was placed on the knees of the operator, sitting with thighs in a horizontal position. The position in which the bird was held was head to the left, tail upwards and feet to the right side of the operator. The tail was pushed forward over the bird's back with the palm of the left hand. The left thumb and forefinger were lightly held against both sides of the vent. The right hand, holding the semen receptacle in the index finger, applied the required massage to the abdomen with thumb and other fingers. On extrusion of the copulatory organ the semen was expelled by a milking action of the left thumb and forefinger, which grasped the organ as deep as possible in the walls of the abdomen. At this critical stage the right hand was entirely free for the collection of semen in the receptacle though before stimulation the heel of the right hand was used against the shanks of the cock to control attempts to escape from the desired position (van Drimmelen, 1945a). For better control a wire loop was constructed to fit the legs of the cock by means of a double hook and supplied with stirrup end for the right foot of the operator [see figure (iii)]

Results:—The percentage successes and the amounts of semen obtained were on the whole better than had previously been the case, as is shown in table 5. On numerous occasions semen was discharged in a short squirt which was due to pressure applied by the fingers and displaced the droplets of semen several feet through the air.

ARTIFICIAL INSEMINATION OF BIRDS.

FIGURE (iii) (a).—The Collection of Fowl Semen.



The collection of semen from fowl cocks by the method of Burrows and Quinn (1935, 1937 1939) with the author's modification for unassisted operators. Note the wire loop with hooks for the legs of the bird, the semen receptacle which is held in position by the right fore-finger only, and the other fingers of the right hand which are employed for massage of the abdomen.

(Photo by Mr. Th. Meyer for the Director of Veterinary Services, Onderstepoort).

FIGURE (iii) (b).—The Collection of Fowl Semen.



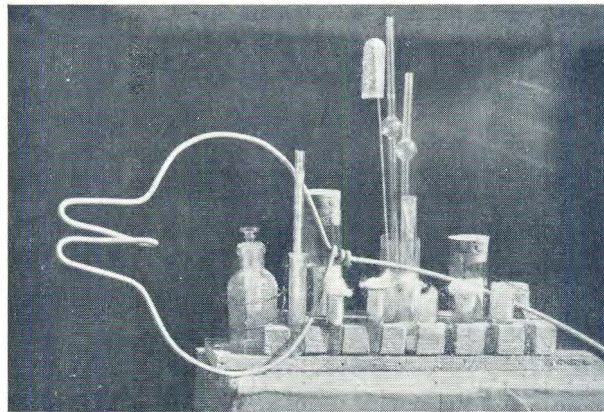
The position of the copulatory organ and the fingers holding the semen receptacle and the vent, during the collection of fowl semen by the author's method.

(Photo by Dr. A. T. Nesor, Bloemfontein).

ARTIFICIAL INSEMINATION OF BIRDS.

The two methods shown first in table 5 were not practised regularly and often enough to reach the same level of efficiency attained by the operator with the last and new method. Whereas the practice of the older methods could be based on previously recorded experience of several workers, the new method had to be developed by trial and error and all the failures are included in the summary set out in table 5. At the conclusion of the first season in which the method was used the level of efficiency reached with six trained cocks was almost 100 per cent. as regards purity of semen. The training of some individuals makes them susceptible to psychological stimuli so that removing a cock from an adjoining cage for semen collection may stimulate ejaculation before the bird is touched.

FIGURE (iii) (c).—Equipment for Semen Collection.



Photograph of rough wooden tube-stand with semen receptacles, pipettes, distilled water and alcohol (65%) for cleaning. Also a wire leg-holder of the author's design.

(Photo by Dr. A. T. Nesor, Bloemfontein).

TABLE 5.
Methods of Semen Collection in the Fowl.

Line.	Method.	Number of Cocks used.	Number of Trials.	Percentage of Trials successful.	Average Amount of Semen produced per successful Trial.	Condition of Semen; Percentage of Samples slightly or badly soiled.
a	Attached receptacle (Parker).....	2	28	35.7%	0.175 c.c.	90%
b	Stimulated ejaculation, bird held by assistant (Burrows and Quinn).....	6	51	39.9%	0.20 c.c.	30%
c	Stimulated ejaculation, bird held by operator (own modification).....	21	292	95.6%	0.471 c.c.	33%
	TOTALS.....	25	371	—	—	—

Discussion.—(Part 1: Semen collection).—The new modification of the method of Burrows and Quinn for the collection of fowl semen, commends itself by virtue of expedience and economy. The results of the few control collections done by established methods, though not done by experienced hands, indicate that the conditions of work in the case of fowls were not such as to produce average amounts of semen equal to that reported by Burrows and Quinn (1937, 1939). This might be explained by the fact that many of the cocks were culls discarded from the breeding pens for various reasons including low fertility; but the permanent confinement in battery cages may also have played a role. This remains to be investigated. The better results in the early morning were apparently due to the relative emptiness of the bowel after the night's rest without feeding. The fact that both pigeons and fowls were successfully restrained for semen collection by an operator single-handed in the position described, suggests that other birds may be subjected to a similar procedure if desired. In any case it seems to be necessary to bear in mind that psychological factors play an important role in artificial semen collection as was illustrated by the experience with male pigeons removed from the loft to the garage for the purpose.

(2) *The first fertilizations obtained with semen injected into the peritoneal cavity of birds.*

With the object of attempting fertilization of eggs with semen injected into the body cavity in the region of the ovary, a study of the topographical anatomy of the region was made for the purpose of selecting instruments and a site suitable for the operation. A pigeon hen was destroyed by severing the cervical medulla, and dissected by removing the sternum and pectoral muscles when fastened in a supine position. This resulted in the opening of the following cavities: both ventral peritoneal cavities, both lateral abdominal airsacs, the pericardial sac and the thoracic airsacs. The tissues between the ovary and the external surface of the body were examined from all aspects. The following was found relevant to the intended operation:—

- (1) the ovary of birds is contained in the dorsal large peritoneal cavity [cavum peritoneale intestinale: Ellenberger and Baum (1939)]. This cavity adjoins the other peritoneal cavities at several points but there is no connection between them.
- (2) the large lateral airsacs in the pigeon extend to the pubic bones posteriorly.
- (3) the posterior processes of the pubic bones in the pigeon are separated from (not fused with) the ischium by the deep narrow obturator notch.
- (4) The liver, heart, lungs, kidneys and large abdominal blood vessels are vital organs surrounding the ovary on the ventral, anterior and dorsal sites.

Several lengths of small-bore needles were inserted at different points in three further post mortem examinations and from the evidence it was established that the most desirable locality for inserting a needle seeking to penetrate to the region of the ovary, was the left obturator notch. The length of needle required was 4.0 cm. for a penetration of 2.5 to 3.5 cm. necessary in the average homing pigeon and the direction was antero-medial, aiming at the right lung, i.e. passing 2 cm. forwards for every 1 cm. to the right of the site of entry. Similar investigations were made in a fowl hen (van Drimmelen 1945a), with comparable findings. The airsacs in the fowl extend less far backwards and

the posterior pubic process is largely fused with the ischium resulting in an obturator foramen which is too far dorsal to be used for intraperitoneal injection. This information was used in planning the following experiments:—

(a) *Pigeons.*

Pigeon hens were inseminated by intraperitoneal injection of semen to see if fertile eggs might be obtained by this method.

EXPERIMENT (3).

Object: To attempt fertilization of pigeon-eggs by the injection of semen into the ovarian region.

Method: Three hens were selected from a loft in which a number had been separated during the moulting period. Two of these had of their own account mated homosexually and had each laid an infertile clutch on dates approximately ten days previously. The eggs of each bird in this pair could easily be identified, as number 61 was an old bird and a purebred Belgian homer of the "Gits" strain, which laid large eggs weighing ± 22 grams each. The other hen, number 375, was a small, year old crossbred, which laid rounder, smaller eggs weighing about 17 grams each. The third bird, a yearling hen, number 544, was mated to a castrated cock in a separate cage, put next to the cage wherein the two hens were kept together. These birds had grain and water *ad lib.* and a mineral supplement of carcass meal with sulphur, salt and a little ferri-saccharate. Small bits of green lettuce were given two to four times weekly.

The first clutch of each hen was incubated to prove infertility. Incubation was done on the tray of a still-air poultry incubator at a 103° thermometer reading 5 cm. above the tray. All subsequent eggs were set in the same way. As soon as possible after the laying of the first egg of each subsequent clutch, i.e. before ovulation of the second ovum, fresh pigeon semen from several males, collected as described in experiment (1) was injected through the abdominal wall by the following procedure:—A small test-tube (capacity 4.5 c.c.) was sterilized by boiling 1 c.c. water in it over a spirits-flame. It was then shaken empty and dried. Whilst cooling, 0.2—0.4 c.c. physiological saline was poured in from a flask kept at room temperature, and when at about 20°—30° C., semen, collected in the meantime from several males in a sterile glass pipette was mixed with the saline. The hen was placed on a table covered with a towel, her left side uppermost and head towards the operator. She was controlled by the left hand, holding wings and tail together. The feathers of the left abdominal pteryla, where these extend behind the thigh, were removed. The bare area of skin was disinfected with methylated spirits. A sterile 5 c.c. glass syringe with metal nozzle (Pretzel and Schultz: Hamburg; No. 13), fitted with a fine sterile, metal needle ("Westprod" serum needle), was partly filled with diluted semen by suction. The point was introduced into the abdominal cavity of the pigeon through the skin, muscle and peritoneum over the middle of the pubic notch. It was then passed in an antero-medial direction to the region of the ovary, a depth of 3 to 3.5 cm. The diluted semen was discharged in two or more localities in that region. [See figure (iv)].

Results: (Note: One of several earlier trials had been successful in producing fertility. Incomplete records prevent consideration of those cases).

FIGURE (iv).—Intraperitoneal Insemination of the Pigeon.



The position of instruments during the operation for intraperitoneal insemination of the pigeon. Note control of the bird with the left hand and the direction of the needle inserted through the skin and tissues over the obturator notch between the ischium and the posterior process of the pubic bone.

(Photo by Dr. A. T. Nesor, Bloemfontein).

The operations and the results of incubation are given in table 6.

Control of incubation was made by setting two pairs of naturally fertilized eggs from pigeons kept in the loft, in the same incubator during the same period. All four these eggs hatched. This fact is an indication that the fowl egg incubator is at any rate not markedly unsuitable for pigeon eggs although it might be far from ideal.

Considering the primitive technique employed, the first results were encouraging. After a study of further literature on the subject of insemination generally, it was evident, that the three injections had contained spermatozoa which had been subjected to temperature shock as well as dilution shock (Milovanov, 1940) though not to ageing *in vitro*.

(b) *Fowls.*

Hens in full production were subjected to intraperitoneal insemination to collect more data on the result of this new operation.

EXPERIMENT (4).

Object: To attempt successful fertilization of fowls' eggs with semen injected into the abdominal cavity of hens.

TABLE 6.
Incubation Results of Pigeon Eggs Laid After the First Attempts at Intraperitoneal Insemination of Birds.

Line.	Number of Bird.	Laying of the First Egg.		Intraperitoneal Injection.		Amount of Semen Injected.	Laying of the Second Egg.			Incubation Results for the Second Egg.
		Time.	Month.	Time.	Day.		Time.	Day.	Month.	
a	61	17.30	Aug.	17.35	23rd	0.025cc.	16.00	25th	Aug.	Infertile.
b	375	17.00	Aug.	17.35	23rd	0.05c.c.	16.00	25th	Aug.	Embryo died on the 5th day of incubation.
c	544	14.00 to 17.00	Sept.	17.00	19th	0.05cc.	18.00	21st	Sept.	Chick dead in shell after 16th day of incubation.

TABLE 7.
The Result of the Second Intraperitoneal Insemination in the Fowl Hen. Bird No. 9.

Item.	NUMBER GIVEN TO EACH SUCCESSIVE DAY AFTER INSEMINATION.																											
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Eggs that were laid.....	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
Size of clutches.....	2						8							1				4			1				4			
Eggs that were proved fertile.....	f	f	f	f	f	f	f	f	f	f	f	f	f	f	f	f	f	f	f	f	f	f	f	f	f	f	f	f
Eggs that hatched.....	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Eggs with chicks dead in shell.....							D										D											
Eggs with dead embryos.....																		d										
Eggs accidentally broken.....											b																	
Chicks killed by manipulation.....																								K				

Method: Three Buff Plymouth pullets (birds number 9, 10, and 11) were placed in single cages of a laying battery. These birds had grown from chicks hatched on the last day of January i.e. very late in the season, from a batch of eggs subjected to injections of colchicine alkaloid (0.000,005 grams) into the egg on the 48th hour of incubation. They were raised in a run separated from cocks and transferred to the battery cages early in September. All had commenced production before this time.

When several weeks in the battery under the conditions described earlier in this chapter, birds number 9 and 10 were found to be good producers, but No. 11 a poor producer. All the eggs laid in the battery were incubated and found infertile.

FIGURE (v).—Intraperitoneal Insemination of the Fowl Hen.



Photograph of one of the first intraperitoneal inseminations performed on a fowl hen. Note the control of the bird only by the wire leg-holder designed by the author and the left hand. Note also the direction of the needle inserted through the skin at the antero-ventral border of the pubic bone.
(Photo by Dr. A. T. Nesor, Bloemfontein).

(i) *The first operation.*—A glass receptacle was made by cutting the lower 3 cm. off a hard glass test-tube of 15 mm. diameter. The semen of White Leghorn Cock No. 6 was collected in this and transferred immediately to a sterile 5 c.c. glass syringe. A long, stout needle was then fitted to the syringe (“Westprod” exploring needle, 11 cm. long and 1.8 mm. in diameter).

Hen No. 9 was taken from the battery, placed on her right side on the knees of the seated operator, with her head towards his body and her legs to his right hand side. Some feathers were removed from the pubic region and the skin cleaned with methylated spirits.

The point of the needle was introduced at the anterior border of the pubic bone immediately ventral to the longitudinal superficial muscles visible through the skin at this point. It was passed through the skin and abdominal wall in antero-medial direction to reach the region of the ovary at a penetration of about 8 cm. and 0.05 c.c. of the semen was discharged in one dose at 19.00 hours on the 5th October [See figure (v)].

During the following five days four eggs were laid, but no trace of fertility could be demonstrated on incubation in any one of them.

(ii) *Second operation.*—The same bird was subjected to an identical operation at 15.00 hours on the 10th October; but the volume of semen discharged was increased to 0.25 c.c. The result is shown in table 7., the day following being designated as day number 1. Twenty eggs were laid in a period of twenty-eight days following the operation. The failure to hatch of the egg laid on the eighth day may be due to muscular contractions stimulated by a dose of pitressin given intramuscularly on the evening of the seventh day. The bird was inadvertently included in an experiment on hatchability and shell texture not connected with the present work, but the shell texture in this case may have been detrimentally affected and may have contributed to the death of the chick in the shell.

Controls.—The experiment was done when the season was already far-advanced but it was possible to collect some parallel results with other methods of insemination and also with other semen and diluted semen. The details and results of these controls are described in the following sequence:

Firstly: *Operation* (iii), the same semen dosed *per vaginam*.

Secondly: *Operation* (iv), intraperitoneal insemination of a poor producer.

Thirdly: A natural insemination case kept under the same conditions.

Fourthly: *Operation* (v), intraperitoneal insemination with semen diluted in albumen.

Fifthly: *Operation* (vi), intraperitoneal insemination with poor quality semen.

Sixthly: *Operation* (vii), intraperitoneal insemination with the standard fully effective dose (0.1 c.c.).

Operation (iii): Bird number 10 was inseminated *per vaginam* by the method of Burrows and Quinn (1939) with 0.25 c.c. fresh semen from the same ejaculate as that used in operation (ii) collected from cock number 6 at 15.00 hours on the 10th October. The result is given in table 8.

Operation (iv).—Bird No. 11 a poor producer, was subjected to intraperitoneal insemination with 0.5 c.c. fresh semen of cock number 6 by the procedure of operation (ii), at 15.00 hours on the 23rd October. The results are tabulated as before: (Table 9).

Natural insemination.—Bird number 12, a Rhode Island Red pullet was acquired and mated to a stud Barred Plymouth Rock cock of known good fertility for the period 10.00 hours to 16.00 hours on one day. This hen was confined in the laying battery and kept under identical conditions to hens 9, 10 and 11. The results of observation of this case are set out in table 10.

Operation (v).—The insemination with semen diluted in albumen was tried when in the absence of any of the recognised “dilutors” (diluents) of fowl semen a diluent was made from fresh, thin egg-white, a liquid occurring naturally on the route of spermatozoa after copulation. It was desired to test the effects of this diluent when introduced directly into the peritoneal cavity. A sample of fresh semen from cock number 6 was mixed with an equal amount of thin egg-white out of an egg that had been laid a few hours before use. At 14.00 hours on the seventh of November 0.5 c.c. of the fresh mixture was injected into the abdominal cavity of hen number 9 by the procedure given with operation (ii). The results are set out in table 11. The “clutch” is here used in the sense of cluster of yolks liberated i.e. twelve ova were liberated in the second clutch in ten days including the three in the premature and double-yolked egg.

Operation (vi).—Intraperitoneal insemination by the technique of operation (i) was carried out with semen of the S.A. Australorp cock number 14, a very old bird of known poor fertility, on hen number 9 at 15.00 hours on the 28th November. Fresh pure semen, 0.25 c.c. in amount, was injected. The results are given in table 12. The fertile egg with live embryo at 13 days incubation had to be placed under a broody pigeon-hen as the incubator was only available to the 22nd of December. The egg failed to hatch and was opened on the 24th day after setting, to show a dead, normal, fully developed, black chick in the normal hatching position.

Note: An observation made in this case was that a smear of the fluid aspirated from the abdominal cavity of hen number 9 at the completion of the sixth operation showed, beside spermatozoa and serous fluid, many polymorphonuclear cells suggesting that some inflamed locality had been penetrated. [cf. operation (v) albumen injected.]

Operation (vii).—The doses used for intraperitoneal insemination here were considered large doses and as Burrows and Quinn (1939) recommended 0.1 c.c. semen as sufficient to provide maximum fertility when given *per vaginam*, this amount of fresh semen from cock number 6 was injected by the technique of operation (i) into the region of the ovary of bird number 9 on the 18th of December at 18.00 hours. No incubator was available until the fifteenth day after this operation when the nine eggs collected during the first 14 days were set. The rest of the eggs were set on the day of collection as in previous cases. The ration of the birds had to be altered on the 20th of December with an effect on production that is clear from table 13.

A discussion of the results obtained in these primary tests is necessary at this stage, as they form the basis on which the present work was planned and carried out.

Discussion [Part (2): first intraperitoneal insemination].

The technique of intraperitoneal insemination, which is entirely new and original, differs from natural processes in several respects. In all species of animals with internal fertilization, artificial insemination has up to the present been carried out by inserting the instruments of insemination through the external genital body-opening. The principle involved here is stated by Walton (1933) viz.: “the ways of nature are preferable to methods which introduce unnecessary deviations with possible unknown influences” (see literature p. 21). It was not possible to find any reference in the literature, on the reaction of spermatozoa to contact with peritoneal fluids, but serum had been tested as a diluent for bull-sperm after inactivation at 60° C. for one hour (Bernshtein, 1933; Grodzirski

TABLE 8.
The Results of Artificial insemination per Vaginam in a good Egg-producer. Bird No. 10.

Item.	NUMBER GIVEN TO EACH SUCCESSIVE DAY AFTER INSEMINATION.																											
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Eggs that were laid.....	E	E	—	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
Size of clutches.....	2	—	—	—	—	—	9	—	—	—	—	—	—	—	5	—	—	—	—	—	3	—	—	—	—	—	—	—
Eggs that were proved fertile.....	—	f	—	f	f	f	—	—	f	—	—	—	—	—	—	—	f	—	—	f	—	—	—	—	—	—	—	—
Eggs that hatched.....	—	H	—	H	H	H	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Eggs with chicks dead in shell.....	—	—	—	—	—	—	—	—	D	—	—	—	—	—	—	—	—	—	—	—	D	—	—	—	—	—	—	—
Eggs with dead embryos.....	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	d	—	—	—	—	—	—	—	—	—	—
Eggs accidentally broken.....	—	—	—	—	—	—	—	b	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

TABLE 9.
The Result of Intra-peritoneal Insemination in a Poorly Producing Hen: Bird No. 11.

Item.	NUMBER GIVEN TO EACH SUCCESSIVE DAY AFTER INSEMINATION.														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Eggs that were laid.....	—	E	—	E	—	E	—	E	E	—	—	—	—	—	—
Size of clutches.....	—	1	—	1	—	1	—	2	—	—	—	—	—	—	—
Eggs that were proved to be fertile.....	—	f	—	—	—	—	—	—	—	—	—	—	—	—	—
Eggs that hatched.....	—	H	—	—	—	—	—	—	—	—	—	—	—	—	—

TABLE 10.
The Results of Natural Mating of Hen No. "12".

Item.	NUMBER GIVEN TO EACH SUCCESSIVE DAY AFTER INSEMINATION.																											
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Eggs that were laid.....	E	E	—	E	E	E	—	E	E	E	E	—	E	E	E	—	E	E	E	—	E	E	E	E	E	—	E	E
Size of clutches.....	2	—	—	—	3	—	—	4	—	—	—	—	4	—	—	—	2	—	—	—	—	5	—	—	—	—	—	2
Eggs that were proved fertile.....	—	f	—	f	f	f	f	—	f	—	—	f	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Eggs that hatched.....	—	H	—	—	—	—	—	H	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Eggs with chicks dead in shell.....	—	—	—	—	D	—	—	—	—	—	—	D	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Eggs with dead embryos.....	—	—	—	d	—	—	d	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Eggs accidentally broken.....	—	—	—	—	—	b	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

TABLE 11.
The Result of Intrapertoneal Injection of Semen Diluted in Fresh Egg-Albumen (0.25 ml. aa) into Hen No. "9".

Item.	NUMBER GIVEN TO EACH SUCCESSIVE DAY AFTER INSEMINATION.																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Eggs that were laid (e = premature ; Dy = double-yolked).....	e	E	—	E	E	E	E	E	E	E	E	Dy	E	—	E	E	E	E	E	E	E
Size of clutches; (i.e., the number of ova liberated).....	2	—	—	—	—	—	12	—	—	—	—	—	—	—	—	7	—	—	—	—	—
Eggs that were proved fertile.....	*	f	—	f	f	f	f	f	f	f*	—	—	—	—	f	—	—	—	—	—	—
Eggs from which chicks hatched normally	—	H	—	H	—	H	—	H	H	—	—	—	—	—	H	—	—	—	—	—	—
Eggs with dead embryos.....	—	—	—	—	—	d	—	—	—	d	—	—	—	—	—	—	—	—	—	—	—

* = Not set.

TABLE 12.
The Result of the Intraperitoneal Insemination of Hen No. "9" with Semen from a Cock of Poor Fertility (Male No. "14").

Item.	NUMBER GIVEN TO EACH SUCCESSIVE DAY AFTER INSEMINATION.																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Eggs that were laid.....	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
Size of clutch.....											19									
Eggs that were proved fertile.....											f									
Egg with chick dead in shell.....											D									
Eggs accidentally broken.....										b										

TABLE 13.
The Result of Intraperitoneal Insemination of Hen No. "9" with 0.1 ml. of Semen from Cock No. "6", (Late Season).

Item.	NUMBER GIVEN TO EACH SUCCESSIVE DAY AFTER INSEMINATION.																																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35			
Eggs that were laid.....	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E		
Size of clutches.....			3		1					1						5						5													2			8
Eggs proved fertile.....			*	f	f				f	f	f	f	f	f	f	f	f	f	f	f	f	f	f	f	f	f	f	f	f	f	f	f	f	f	f	f	f	
Eggs that hatched.....																																						
Chicks dead in shell.....																																						
Eggs with dead embryos.....																																						
Accidentally broken.....																																						

* = Not set.

and Marchlewski, 1935; see p. 16). Parker (1931) referred to the fact that active sperm had been found abundantly about the ovaries of both birds and reptiles (see p. 29). It is clear, however, that spermatozoa are generally believed to emerge from the cranial end of the fallopian tube after natural coitus for a limited period of time, but when they have reached this site they soon disintegrate to be resorbed (see p. 30). By placing semen directly in the field of activity the natural process was altered by eliminating firstly the time spent in the oviduct, secondly energy consumed during this journey and thirdly the dilution of the semen by the secretions in the oviduct. Some contents of the semen, which might not usually reach the upper end of the genital tract under natural circumstances e.g. sperm serum or *abnormal* sperm cells could, however, come to this locality as the result of applying the new method.

The fact that fertilization was achieved by the intraperitoneal injection of semen suggests that the experiences of sperm cells in the oviduct are not essential for fertility. It might be suspected, that in the few cases mentioned the point of the needle had entered the oviduct. This must be considered highly improbable as it was not possible to penetrate any of the abdominal organs excepting the liver, in freshly killed pigeons, even with the sharpest needle, unless a vigorous jerk was applied. The new technique of intraperitoneal insemination offers a field for investigation of factors connected with fertilization and development e.g. the time, locality and method of sperm storage in the organs of the hen.

Unfortunately in birds the female is heterogametic for sex and not the male, so that avian sperm cannot be of value for work on sex-ratio influence.

The results of the three cases of intraperitoneal insemination in pigeons and the eight cases in fowl hens reported in this part will now be considered in the sequence in which they were carried out:—

(a) *Pigeons*.—The conditions under which experiment (3) was done were not very favourable for good results. The main difficulty was the smallness of the amounts of semen that had to be handled, which, with the general utility size of the instruments that had to be used, required excessive dilution and hindered attempts to avoid the effects of dilution shock and temperature shock on the spermatozoa. It is probable that clumps of sperm cells remained together, during the few minutes between dilution and discharge, in the region of the ovary and thus, to some extent, escaped damage from the sudden contact with large quantities of foreign fluid. Both fertile eggs failed to hatch however, whereas it was shown that naturally fertilized pigeon eggs incubated under similar conditions actually hatched.

(b) *Fowls*.—The first operation on fowls was done with a small amount of semen because it was feared that peritonitis would easily result from the bacterial contaminants, which the semen was believed to have acquired during emission from the male organ. However, there was no sign of any detrimental effect on the hen and production was continued normally although only infertile eggs were obtained. The second operation with a five times larger dose was equally harmless and exceptionally successful in respect of fertility as well as hatchability of the eggs that followed. In this case spermatozoa, injected directly into the ovarian region of a hen in very good production, fertilized 100 per cent. of the sixteen eggs laid during twenty-four days after the day of operation and set on the day of laying. On the first day following the day of operation, no egg was laid so that the first fertile egg was laid about 41 hours after insemination and the last fertile egg on the 24th day (i.e. 567 hours after, to the nearest hour). Recorded observations give six eggs (Chappellier, 1914), eleven eggs (Curtis and

Lambert, 1929), and fourteen eggs (Nicolaidis, 1934) as the maximum to be fertilized in the fowl after separation from the cock. Hatchability was maintained at the same high level throughout the 24 days; chicks hatched out of eggs laid on the second, third, fifth, sixth, seventh, ninth, tenth, twelfth, fifteenth, nineteenth, twentieth, twenty-second and twenty-fourth day following the day of insemination. In this respect the case differs from the average found after removal of the cock, as Nalbandov and Card (1943) showed that normal chicks could only be obtained from eggs laid during twenty days after removal of the cocks from the runs, counting the day of removal as the first, which implies that his period includes the time up to the nineteenth day after the last fertilization. Fertile eggs laid between the nineteenth and the 32nd day after coitus have in the past consistently failed to complete embryonal development. (Barfurth, 1896; Crew, 1296; Nalbandov and Card, 1943).

The third operation done as a control with an equal amount of semen from the same ejaculate inseminated *per vaginam* into a similar good producer, showed results comparing with the observations of the authors mentioned, viz.: (1) fertility lasting three weeks but fertile eggs laid in the second and third week failed to hatch and (2) a marked drop in percentage of eggs fertile in the second and third weeks. The fourth operation had results expected from the experience reported with the older methods of insemination, i.e. poor production was associated with poor fertility. The naturally inseminated hen showed results which again conform in general with data on record, viz. fertility that lasted thirteen days and the egg laid on the ninth day was the last fertile egg to hatch. Here also the percentage fertility dropped after the first week.

The diluent used in connection with the fifth operation was recommended by Griffini (1938) and others, and thought useful on the following grounds: (1) it was a natural diluent closely resembling fluid contents of the oviduct with which sperms come in contact during passage after normal coitus; (2) it was easily obtained sterile from a fresh egg. The result is not considered a success as a derangement occurred in the regular sequence of ovulation on the tenth and twelfth days, a fact that suggests damage done to the ovary, perhaps from traumatic injury by the point of the needle or from inflammatory changes caused by egg albumen. This latter suggestion is supported by the finding in the smear of fluid aspirated after operation (vi). Encapsulated bits of egg material are often seen in the abdominal cavities of fowls on routine post mortem examinations. Apart from this fact the results of this intraperitoneal insemination conform with results from natural copulation, viz.: fifteen-day fertility with infertile eggs in the second week only. The fact that the last egg proved fertile and hatched normally must, however, be specially noted.

The sixth operation was followed by only one fertile egg which was, peculiarly, the egg laid on the eleventh day after insemination. With the present knowledge of the physiology of fertilization it is impossible to explain how such a result could have been brought about, as the fact that it was preceded by nine infertile eggs, laid since the injection of semen, is extremely exceptional (Munro, 1938; Nicolaidis, 1934). The seventh operation produced a fertility in many respects equal to that caused by operation (ii) i.e. the egg laid on the 24th day after insemination was proved fertile and hatched. The occurrence of infertile eggs during the fertile period and the failure of some embryos to complete development may have been caused by the keeping of some of the eggs before setting and by the seasonal influence of the late summer when the test was carried out, rather than by the relatively smaller dose of semen used for insemination. On the whole the data collected from the work described in this part, were sufficiently encouraging and informative to justify further investigation.

(3) *The effect of intraperitoneal insemination on the Production and Health of Birds.*

To ascertain the consequences of intraperitoneal injections of semen in hens, operations as described in experiments (3) and (4) were carried out in a number of pigeons and fowls. Observations were made on the production and health of the birds, before and after the injections.

(a) *Pigeons.*—The data obtained from pigeons do not permit an easy summary on account of the marked individual variations, the small number of eggs that they are able to produce and the difficulties of incubation. Results are set out in table 14. The final rapid growth stage of pigeon ova commences six days before laying of the egg formed around it. It was therefore taken to occur during the period 44 to 144 hours prior to laying as ovulation precedes oviposition by about 44 hours (Riddle and Behre, 1921). The operations that were carried out during this period were considered separately as it was felt that large ova were particularly vulnerable to the needle-point introduced into the ovarian region.

During the period 44 hours to 144 hours after operation normal eggs were laid in 46 cases, an abnormal egg was laid once, and five times pigeon hens failed to lay the second egg of their clutch. In three cases hens failed to lay within the period kept under observation after an intraperitoneal injection. Two of these pigeon hens commenced to moult and they were eventually returned to the loft, but the third, No. 375, showed a diarrhoea with loss of condition. She was killed for post mortem examination and a large sequestre was located in the abdomen and this was found to contain a rusted wire needle pass-through, which no doubt had been accidentally transmitted during operation.

(b) *Fowls.*—The influence of the intraperitoneal insemination method on fowl hens will be examined under two headings: (i) production and (ii) health.

(i) *Production.*—A summary of the data collected is given in table 15. This includes the information on all 117 intraperitoneal inseminations performed in the first series. Consequently some of the records overlap, so that the same egg may, for instance, be shown as laid three days after the one insemination and again as laid six days before the next insemination of the same hen. The following points should therefore be borne in mind when reading table 15.

- (i) Of the 117 consecutive intraperitoneal inseminations here summarized, 42 or 36 per cent. were performed within ten days of a previous insemination on the same bird whether the same or another method of insemination was used.
- (ii) In 14 of the 117 cases, inseminations by other methods were performed simultaneously on the same hens, and these may be responsible for the effects produced.
- (iii) In 13 of the 117 cases, hens were moved, handled, or otherwise dealt with in such a manner that the change in production may be attributed to causes other than the act of intraperitoneal insemination.
- (iv) In 64 of the 117 cases not one of the subsidiary mentioned in (i), (ii) and (iii), was operating, one or more being present only in the 53 remaining cases.

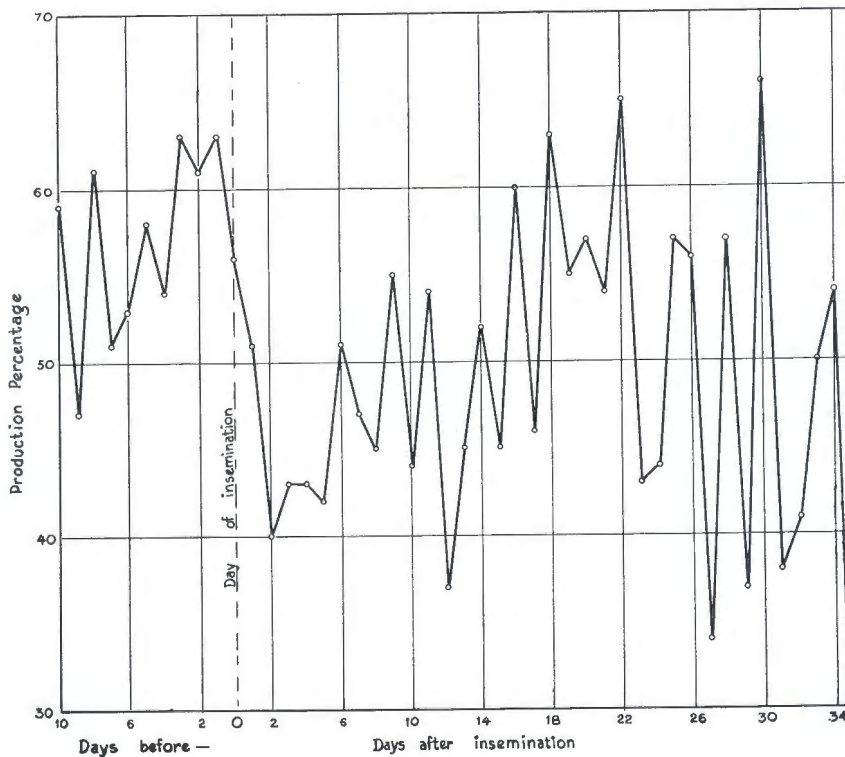
TABLE 14.
The Effect of Intrapertoneal Insemination on the Egg-production and Health of the Pigeon Hen.

Line.	Item.	NUMBER IDENTIFYING EACH BIRD.													Totals for the 14 Birds.	
		544.	61.	375.	37.	503.	304.	303.	299.	222.	p9.	504.	244.	63.		688.
a	Number of intraperitoneal inseminations performed	4	15	5	5	2	5	1	3	3	3	6	2	4	4	62
b	Number of cases in which eggs were laid after intraperitoneal insemination.	4	14	4	5	2	5	1	3	3	3	5	2	4	4	59
c	Number of cases in which normal eggs were laid 44 to 144 hours after insemination.	4	11	4	4	2	4	1	0	2	3	2	2	4	3	46
d	Number of cases in which abnormal eggs were laid 44 to 144 hours after insemination.	—	—	—	—	—	—	—	—	—	1	—	—	—	—	1
e	Number of cases in which no second egg was laid following insemination performed:— More than six days before the first egg was laid.	—	—	—	—	—	—	—	1	—	—	1	—	—	—	2
f	44 to 144 hours before the first egg was laid.	—	—	—	—	—	—	—	—	1	—	—	—	1	—	3
g	After the first egg of the clutch was laid.	—	1	—	—	—	—	—	—	—	—	1	—	—	—	2
h	Number of cases in which pigeon hens stopped laying altogether after an intraperitoneal insemination.	—	1	1	—	—	—	—	—	—	—	1	—	—	—	3
i	Number of sick birds killed and examined at <i>post mortem</i> .	—	—	1	—	—	—	—	—	—	—	—	—	—	—	1

Twenty-eight hens were used in this series and each "Hen-day", i.e. a single hen observed for a single day, was counted as one observation, 3421 observations being used for these records. This figure represents the total observations made in the first series of inseminations plus a few of the "Hen-days" which overlap in successive insemination cases. The effect of the intraperitoneal insemination operation is illustrated in Graphs C, D, and E. All observations ("Hen-days") are included and no results are discarded here on account of the operation of subsidiary factors at the time of insemination by the intraperitoneal method. Graph E is specially given because in the fowl the final rapid growth stage of ova takes 10 days. (See lit. page 26). (Warren and Conrad, 1939.)

The results of the different subsidiary factors mentioned were considered to constitute a probable interference. For this reason a comparison was made in table 16 and in Graph F, to show that the results in the 64 uncomplicated cases out of the total of 117 intraperitoneal inseminations, were in general conformity with the gross results as well as with the control results.

GRAPH C.—The daily percentage of total egg-production of twenty-eight fowl hens, on the days between the tenth day prior to, and the fifth week after one-hundred-and-seventeen (117) intraperitoneal inseminations.



Graph C shows the reduced egg-production on the day of insemination and on the two days following. Note that the recovery between the sixth and eleventh day is not continued until the seventeenth day after the operation. The great variation of the daily percentage production in the fifth week is due to the small number of cases observed to that extent.

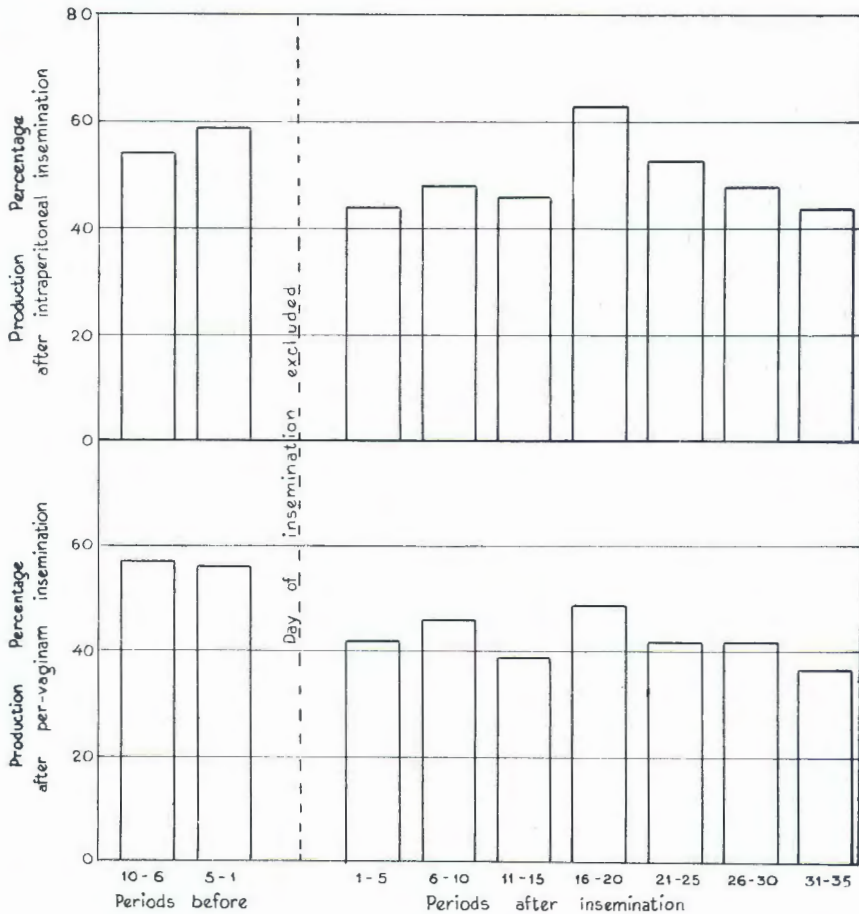
ARTIFICIAL INSEMINATION OF BIRDS.

TABLE 15. The Eggs laid by Fowl Hens, before and after 117 Consecutive Intra-peritoneal Inseminations.

Line.	Description of Day.	Number of Observations given in "Hen-days".	Number of Days on which an Egg was laid.	Production Percentage.	Number of Cracked Eggs.	Number of Premature Eggs.
a	Days before insemination.....	10	54	59%	2	Nil
b		9	43	47%	2	1
c		8	94	61%	2	1
d		7	98	51%	Nil	1
e		6	101	53%	Nil	Nil
f		5	104	58%	2	Nil
g		4	104	54%	Nil	1
h		3	106	63%	1	2
i		2	106	61%	Nil	1
j		1	107	63%	1	1
k	Day of insemination.....	0	117	56%	2	3
l		1	117	51%	2	4
m		2	116	40%	1	4
n		3	116	43%	2	4
o		4	115	43%	Nil	Nil
p		5	114	42%	1	1
q		6	112	51%	Nil	1
r		7	108	47%	1	2
s		8	100	45%	1	Nil
t		9	99	55%	Nil	Nil
u	Day after insemination.....	10	99	44%	1	2
v		11	95	54%	1	Nil
w		12	90	37%	Nil	Nil
x		13	83	45%	1	Nil
y		14	79	52%	Nil	Nil
z		15	76	54%	1	Nil
aa		16	67	60%	2	1
bb		17	63	46%	Nil	Nil
cc		18	59	63%	Nil	1
dd		19	56	55%	1	1
ee	20	55	57%	3	1	
ff	21	54	54%	Nil	Nil	
gg	22	51	65%	1	Nil	
hh	23	48	43%	Nil	Nil	
ii	24	45	44%	Nil	1	
jj	25	39	57%	Nil	Nil	
kk	26	39	57%	1	Nil	
ll	27	39	34%	Nil	Nil	
mm	28	37	57%	1	Nil	
nn	29	32	37%	Nil	Nil	
oo	30	30	66%	Nil	Nil	
pp	31	21	38%	Nil	Nil	
qq	32	17	41%	Nil	Nil	
rr	33	12	58%	Nil	Nil	
ss	34	11	54%	Nil	Nil	
tt	35	8	25%	Nil	Nil	
uu	TOTALS.....	3,421	1,747	—	33	32

The control inseminations which were performed by methods familiar to workers elsewhere, were done in birds kept under identical conditions and during the same season as the experimental operations. The results are compared in parallel columns in table 16, being divided in similar five-day periods to those used in the construction of Graphs D and F. The hens kept under observation for the full 35 days after insemination, were in general not the best producers, as the latter were often used for further experiments before the 28th day. This fact must be kept in mind in the interpretation of the results of the last few periods in table 16 and in Graphs C to F.

GRAPH D.—The percentage egg-production grouped in five-day periods before and after intraperitoneal insemination (experimental) and insemination *per vaginam* (control), in separate, comparable columns. (See Table 16).



Graph D shows the similarity of effect on egg-production caused by the intraperitoneal operations and the inseminations *per vaginam*.

Table 16 shows the percentage of days on which eggs were laid during each of two five-day periods before and seven five-day periods after the day of insemination in respect of the total 117 intraperitoneal inseminations [columns (2)

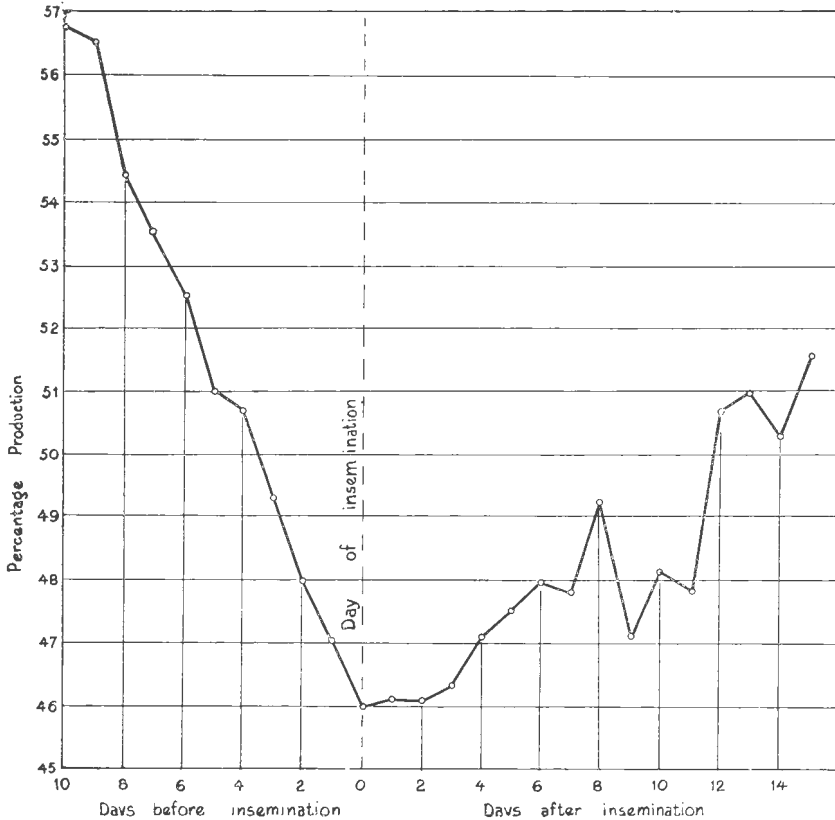
TABLE 16.
The Effect of Insemination in Fowl Hens, on the Rate of Egg-production after the use of Different Methods.

Line.	Five-day Periods before and after Insemination.	INTRAPERITONEAL INSEMINATION.				Per <i>vaginam</i> INSEMINATION.			NATURAL COITUS.	
		Total Cases.		Uncomplicated Cases.		Total Cases.		Total Cases.		
		Number of Observations.	Percentage Production.	Number of Observations.	Percentage Production.	Number of Observations.	Percentage Production.	Number of Observations.	Percentage Production.	
	Column (1)	Column (2)	Column (3)	Column (4)	Column (5)	Column (6)	Column (7)	Column (8)	Column (9)	
a	10-6 days before.....	475	54%	239	51%	101	57%	30	67%	
b	5-1 days before.....	527	59%	269	56%	110	56%			
c	1-5 days after.....	598	44%	312	43%	110	42%	110	56%	
d	6-10 days after.....	518	48%	288	48%	97	46%			
e	11-15 days after.....	423	46%	223	46%	82	39%	88	67%	
f	16-20 days after.....	292	63%	154	53%	65	49%			
g	21-25 days after.....	225	53%	117	48%	57	42%			
H	26-30 days after.....	166	48%	86	41%	38	42%	61	66%	
i	31-35 days after.....	69	44%	32	41%	19	37%			

Table showing the percentage of days on which eggs were laid during each of two five-day periods before and seven five-day periods after the day of insemination in respect of 117 intraperitoneal inseminations, 64 selected intraperitoneal inseminations in which no subsidiary influences were acting, twenty-two control inseminations *per vaginam* and eleven natural matings.

and (3)], 64 selected intraperitoneal inseminations in which no subsidiary influences were acting [columns (4) and (5)], 22 inseminations *per vaginam* and in respect of 11 natural copulations which latter had to be grouped in ten-day periods on account of smaller numbers of observations.

GRAPH E.—The percentage egg-production for ten-day periods following each day between the tenth day before and the fifteenth day after intraperitoneal insemination; i.e., all eggs from ova advanced past the initiation of the final rapid growth stage are considered on each day.



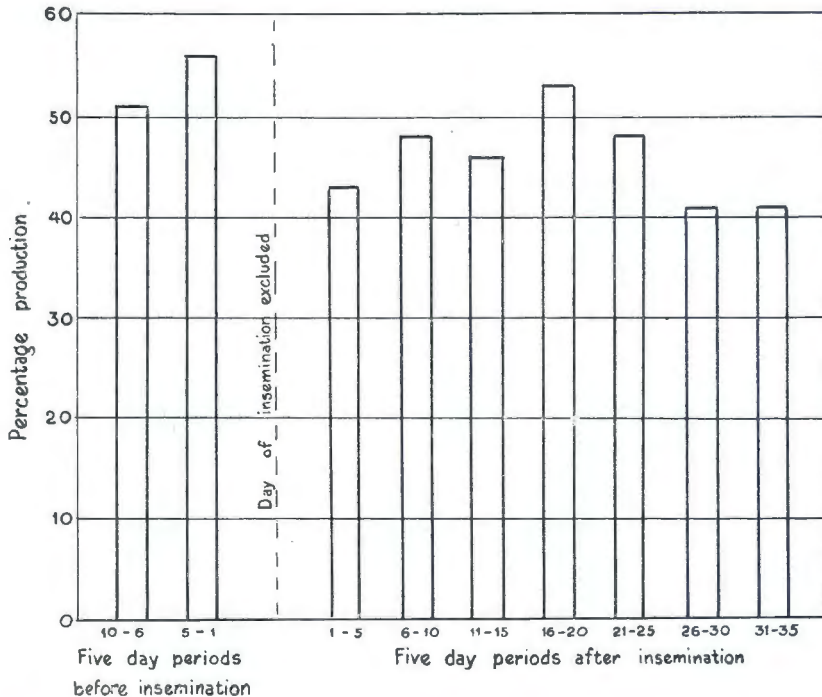
Graph E shows the regular drop in the total egg-production for the ten-day periods after each day from the tenth day prior to insemination, down to the day of insemination. The recovery is found during the ten-day periods following the fourth to the eighth day after the operation, and again from the twelfth day on, the second drop reflecting the lowered production during the ten-day periods following the ninth to the eleventh day after insemination.

By discarding all intraperitoneal inseminations in which the records were not complete for the whole period between the tenth day prior to the day of insemination and the tenth day after the day of operation, sixty-seven cases were selected to demonstrate the effect of intraperitoneal insemination (Graph G).

All the 67 cases in which the records of egg production were complete for 10 days before to 10 days after the day of insemination (and in which the eggs were collected), were used in the data for compiling Graph G. Thus whereas 48 of the cases showed a 50 per cent. or higher production before (see continuous

lines Graph G), this level was maintained by only 34 during the ten days after the operation. At the barrier of 60 per cent. (see broken lines Graph G) production during the ten days prior to artificial insemination, hens were laying more in 36 cases or in more than half the cases, but in the ten days after, only in 22 cases, or in just under one third of the cases the birds were able to lay seven eggs or more after the operation.

GRAPH F.—The percentage egg-production grouped in five-day periods before and after sixty-four selected intraperitoneal inseminations, performed at a time and under circumstances which would exclude as far as possible, all influences from subsidiary factors likely to effect the results.



Graph F shows that the effect of intraperitoneal insemination, even in cases selected to exclude additional influences, was similar to the control results.

Several *individual cases* are of special interest because of change in egg-production that was manifest within a matter of hours after intraperitoneal insemination:—

- (i) In six cases hens failed to lay during ten days after insemination although they produced respectively seven, four, three, two, two and one egg before the operation in a ten-day period.
- (ii) Abnormal eggs (premature) were observed before and after intraperitoneal insemination at a strikingly different frequency as shown in table 17. Sixteen premature eggs were found in ten days following the day of insemination as against eight during an equal period of time before. Although the observations during the ten days after were more numerous than during the ten days before (see table 15: 1096

against 1002), it is significant that four abnormal eggs could be found of the first two days after the inseminations, and three on the day of operation, whereas on no other day of the whole series more than two premature eggs were seen.

(iii) Double yolked eggs in the same 3421 Hen-days observed were found as described in the following list:—

- One on the ninth day prior to intraperitoneal insemination.
- One on the second day prior to intraperitoneal insemination.
- One on the day of intraperitoneal insemination.
- One on the first day after intraperitoneal insemination.
- One on the ninth day after intraperitoneal insemination.
- Two on the tenth day after intraperitoneal insemination.
- One on the twelfth day after intraperitoneal insemination.
- One on the eighteenth day after intraperitoneal insemination.
- Two on the nineteenth day after intraperitoneal insemination.

GRAPH G.—The number of cases in which each of the possible numbers of eggs laid during the ten days prior to insemination coincided with each of the possible numbers of eggs laid during the ten days after insemination.

		No. of Eggs laid during the 10 days before insemination.																		
		0	1	2	3	4	5	6	7	8	9	10								
No. of Eggs laid during the 10 days after insemination.	0		1	2	1	1			1				33	45						
	1					1	2	1	2						33	45				
	2				1			1		1							33	45		
	3							1	2										33	45
	4							1		1										
	5						3	1	4	4		1	33	45						
	6						2	3	4	2	1				33	45				
	7		1			1	2	2	2	4	2						33	45		
	8							2	1		1	1							33	45
	9						1				1									
	10									1			33	45						
												Total: 67 Cases.								
19						48										34	22			
31						36												34	22	
Total : 67 Cases.																				34
													34	22						
												34			22					

Graph G shows that the 67 cases in which the records were complete, included forty-eight with six eggs or more during the ten days before, but only thirty-four with that number during the ten days after; and again thirty-six with seven eggs or more during the ten days before, but only twenty-two with that number after the operation.

TABLE 17.

The Premature Eggs observed in 3421 "Hen-days" between the tenth day before and the thirtyfifth day after 117 Intra-peritoneal Inseminations.

Line.	Number of Premature Eggs observed on one day.	Days, in respect of the day of Insemination, on which such number of Premature Eggs were observed.			Total Number of Premature Eggs Recorded.
		Days before the day of Operation.	The same day as the day of Operation.	Days after the day of Operation.	
a	Four.....	—	—	1st and 2nd	8
b	Three.....	—	The same day	—	3
c	Two.....	2nd	—	4th, 7th and 10th	8
d	One.....	1st, 3rd, 4th, 7th, 8th and 9th	—	5th, 6th, 16th, 18th, 19th, 20th and 25th	13
	Total number observed	8	3	21	32

Controls.—A limited number of control cases, could be observed under identical environmental conditions. The control methods of insemination were the two practised up to the present in other countries:

(i) Artificial insemination *per vaginam* (Quinn and Burrows, 1936; Burrows and Quinn, 1939)

(ii) Natural Copulation.

(i) Insemination *per vaginam*: Twenty-two inseminations *per vaginam* were recorded, eighteen with complete records from ten days before to ten days after the insemination. Although the number is small the trend of the production curve can be seen in Table 16: and in Graph D.

A premature egg was found on the 9th day before, and on the 3rd, 7th, 10th, 12th and 20th day after these inseminations.

(ii) Natural copulation was tested on eleven occasions. Only twice were the results from a single coitus; the others were from one-day matings and necessitated considerable disturbance of the birds.

The 289 observations made are presented in the last column of table 16.

N.B.—The environmental factors in all work of this nature, play a role which is often extremely difficult to assess so that comparisons of local results with other data become very unreliable. Hence the local results of *per vaginam* insemination and natural copulation are given.

(ii) *Health.*—Table 18 shows the detrimental effects from the three methods of insemination employed in this series, in the form of a brief summary.

TABLE 18.
The Ill-effects Observed in the First Series of Inseminations.

Line.	Method of Insemination.	Number of Cases of Insemination.	Number of Cases followed by complete stoppage of production for at least Ten Days.	Number of Cases with symptoms of illness, e.g., Diarrhoea, Anaemia.	Number of Sick Birds killed for Investigation.	Number of Cases followed by Death.	Post Mortem Findings.
a	Intraperitoneal Insemination	117 (in 28 hens)	6	3	2	1	Haemorrhage once and peritonitis twice.
b	<i>Per vaginam</i> insemination	22 (in 13 hens)	5	1	0	1	Haemorrhage.
c	Natural copulation. . . .	11 (in 10 hens)	0	0	0	0	--

Though stoppage of production is not necessarily a sign of illness, it is mentioned in table 18 because of the possibility that intraperitoneal injections of material into the region of the ovary may produce internal organic deviations.

A short history of each fatal case is given here:

- (i) Bird number 31, was a White Leghorn pullet which died twelve hours after the fifth intraperitoneal injection of semen performed on her. Post mortem examination revealed that the very thin long needle used in this last operation (0.6 mm. × 100.0 mm.) had not remained straight, but had been deflected dorsally at the point, being very flexible, and had penetrated the large abdominal blood vessels, leading to fatal internal haemorrhage. The clots that could be collected weighed together about 40 grammes.
- (ii) Bird number 43, was a White Leghorn pullet which stopped laying after the sixth intraperitoneal insemination performed on her. Only a yolk and some albumen was expelled about two hours after the operation. She stopped feeding on the fifth day, lost weight rapidly and developed cyanosis. She was killed on the sixth day and at post mortem examination, peritonitis and broken and partly resorbed yolks were found in the abdomen. Emaciation was considerable. There was no history of impure semen being used for insemination.
- (iii) Bird number 50, was a two year old S.A. Black Australorp hen, which stopped laying after having been isolated three days and inseminated on the second day by the intraperitoneal method. About two days after the operation she developed a severe diarrhoea and started losing weight. She was killed on the ninth day and found to suffer from peritonitis but appeared to be recovering as all the organs seemed normal. The hen never stopped feeding.
- (iv) Bird number 19, was a Rhode Island Red pullet which laid her last egg just prior to her fourth insemination *per vaginam* [method of Burrows and Quinn, (1939) and Quinn and Burrows, (1936)]. A premature membranous egg was expelled three days later. She lost weight, was very anaemic and was destroyed *in extremis* on the twelfth day for post mortem. Examination showed a rupture of the liver with extensive haemorrhage and partly resorbed blood clots. Emaciation was advanced.

In connection with the health aspect attention must be drawn to the observation in part (2) at the time of the operation No. (vi) (see page 53) where polymorphonuclear cells were aspirated from the peritoneal cavity 21 days after injection of semen diluted in albumen. The large number of these cells must be considered abnormal and although no break in production occurred a lesion of inflammatory nature is suspected to have been produced.

Discussion.—The evidence collected shows that as a rule no ill-effects are caused by intraperitoneal insemination in the pigeon and fowl. Single-egg clutches occasionally occur in the pigeon loft, particularly if the eggs of a female are often removed, so that, instead of laying once per month, as in the case of rearing the squeakers, she is encouraged to produce a clutch twice or three times per month. The fact that two single-egg clutches occurred with pigeon hens not operated upon within 144 hours of laying, markedly reduces the significance of the five single-egg clutches found after the inseminations. The sixty-two operations caused no illness or mortality, excepting the case due to accidental introduction of a foreign body.

The data submitted in this part on fowls, show that a definite effect on production generally, was observed after both experimental and control inseminations. (Table 16). The daily percentage production before and after intraperitoneal insemination as shown in Graph C, presents excessive variation outside the limits of the sixth day before to the twenty-first day after the operation on account of the limited observations outside this period. The reduction of egg-production from 63 per cent. to 56 per cent. on the day of insemination is unlikely to be exclusively the result of the operation, as an egg in the hen's uterus will be laid on the day due as a rule, although laying may be delayed for some hours. This finding suggests that factors which started operating before the day of insemination e.g. a change from run to battery-cage, had an influence on the results. The drop to 51 per cent. on the first day after, and to 40 per cent. the second day after insemination may have been due to inhibition of ovulation in consequence of manipulation and injection of semen into the ovarian region. The production level showed no marked recovery from this level until the sixth day, so that ovulation was interfered with for at least five days.

By taking together the production of five-day periods as was done by Nalbandov and Card, (1943) (see lit. p. 31) the trend in variation of production is demonstrated better (see Graph D). There was a sharp drop in production during the first five-day period after the day of insemination; a partial recovery followed between the sixth and tenth day, but this was not sustained as a slight drop occurred again between the eleventh and fifteenth day. Full recovery was shown to have been reached only after the sixteenth day, and the pre-operation level was then exceeded. Such an increased production after a period of interference could have been expected from general poultry experience. After retarded laying due to adverse weather or feed conditions, hens often recover under improved conditions to surpass their estimated probable percentage of daily egg-production for a time. This was well illustrated at the Glen College of Agriculture in the Central Laying Test 1944, when an acute mealie shortage during the first 3 months interfered with the normal rationing. The resultant low egg-production was followed by a very high production when the ration was restored, giving a normal annual total. The extra eggs compensated for the initial loss. The partial recovery before the tenth day, and the slight reverse after the tenth day following on the day of insemination may be associated with ovarian physiology, viz.: the stoppage in the initiation of the final rapid growth stage in the development of ova may have been greater than the retardation in the rate of ovulation of ova which had already commenced the final rapid growth stage. This view is supported by the findings shown in Graph E, where the total egg production for ten days after each day is depicted for each of the days between the tenth day before, and the fifteenth day after, insemination. Here the second smaller drop in production is seen to deviate from the regular curve of recovery, between the eight and the eleventh day i.e. fewer eggs were laid between the eighth and 21st day, than between the fourth and seventeenth day after intraperitoneal insemination.

Under the same conditions, however, *per vaginam* insemination and natural mating had strikingly similar results (see Table 16). This is important as no other control data are available on artificial insemination of poultry in the dry hot South African climate. This finding suggests, that the disturbances necessitated by insemination in this series, rather than the method of insemination, was responsible for most of the effects on production observed here. Such a view is also supported by the fact that the 64 selected cases, (in which