HAS OVOTHERAPY, AS NOW PRACTICED, AN EXPERIMENTAL BASIS? *

ROBERT T. FRANK, M.D.

NEW YORK

INTRODUCTION

Our knowledge relating to the function of the corpus luteum is limited to a few isolated and disjointed facts. The minority are based on reliable experimental data; the majority are insecurely founded on clinical observations, which permit of widely differing interpretation.

Even a cursory study of the corpus luteum under the microscope will convince an unbiased observer that a structure which is stamped with all the hallmarks of an extremely active gland must possess at least some, and probably important functions.

As Fraenkel has shown, the corpus luteum is widely distributed in the mammalian series. It should be classed among the glands of internal secretion, enjoying the unique distinction of being transitory, appearing with each cycle of rut or menstruation, and persisting during the major part of gestation.

The voluminous literature dealing with the corpus luteum is readily accessible.¹ In this connection only the following articles need be referred to. Fraenkel² has shown that, in most instances, the nidation of the ovum is prevented in the rabbit, if the ovary or the corpus luteum is ablated within seven days of fertilization. The doubts cast on this observation by Daels³ and other investigators are based on the fact that the operation required to remove or destroy the corpora lutea may have sufficed to interrupt the pregnancy. This Fraenkel, relying on his control experiments, categorically denies. Leo Loeb⁴ reported in 1909 that amitotic nuclear proliferation was observed in the surface epithelium of the rabbit's uterus on the fifteenth day after the formation of the corpus luteum. No such changes were noted at an earlier period (three and one-half to five and one-half days).

* From the Department of Pathology of Columbia University, College of Physicians and Surgeons.

1. Fellner, O.: *Med. Klin.*, 1906, ii, 1100; Villemin: *Le corps jaune considéré comme glande à sécrétion interne de l'ovaire*, Paris, 1908, Octave Doin.

2. Fraenkel: *Arch. f. Gynäk.*, 1903, lxxviii, 438.

3. Daels: *Surg., Gynec. and Obst.*, 1908, vi, 153.

4. Loeb, D.: *Arch. f. Entwcklungsmechn. d. Organ.*, 1909, xxvii, 463.

Bouin and Ancel,⁵ on the other hand (1910), assert that well-marked epithelial changes occur soon after the rupture of the ovarian follicle, and that these alterations correspond accurately in time with the development of the corpus luteum, and are summarily cut short or prevented by the removal of this body.

These few, and somewhat contradictory observations, which, here, are not reported in strictly chronological order, included practically our entire knowledge of the physiological action of the corpus luteum, until Leo Loeb⁶ (1906-1910) in a large and most convincing series of experiments, has greatly advanced our knowledge by demonstrating that the corpus luteum secretion "sensitizes" the uterine mucous membrane of the guinea-pig and of the rabbit in such a manner that it responds to traumatic stimuli (these may consist of the lytic action of the early ovum, or of the coarser experimental stimuli of incision) by marked proliferation of the submucosal connective tissue. Incisions into the uterus, made from two to nine days after coitus or after rut (in both cases these processes correspond to the rupture of the follicle and to the beginning formation of the corpus luteum) are followed by the appearance of multiple tumors along the line of incision. These tumors attain the size of a pea or even of a hazelnut, enlarge slowly for about ten days, and then as slowly regress. Removal of the entire ovary or of the corpus luteum prevents the appearance of the growths. The deciduomata, as Loeb denominates these transitory tumors, also develop in portions of uteri, transplanted to other parts of the body of the same animal and, though to a lesser degree, into other animals of the same species, who are in a corresponding period of rut.

Microscopically the deciduomata correspond to the maternal (decidual) part of the placenta, differing somewhat in type in the guinea-pig and in the rabbit, as should be expected from the difference of the deciduæ of these two species under normal conditions. In both, the salient features are an enormous proliferation of the connective tissue stroma, immediately beneath the epithelium and about the uterine glands. The tissues assume an exaggerated decidual character; the nuclei of the stroma enlarge and show mitoses; the cell body becomes plump and contains glycogen; the endothelial cells of the vessels enlarge and show division, and, at least in the guinea-pig, the surface epithelium contains large

5. Bouin and Ancel: *Jour. de physiol. et path. gén.*, 1910, xii, 1.

6. Loeb, L.: *The Production of Deciduomata*, *Jour. Am. Med. Assn.*, 1908, 1, 1897; *The Experimental Production of the Maternal Placenta*, *ibid.*, 1909, lii, 1471; *Arch. f. Entwicklgsmechn. d. Organ.*, 1909, xxvii, No. 1.

giant-cells and cell-complexes. The wider general significance of Loeb's researches cannot be entered into in this connection.

To recapitulate: Fraenkel has shown that the corpus luteum plays an important, probably an essential part, in determining the nidation of the ovum. Loeb and later Bouin and Ancel have called attention to the finer histological changes, which, under the influence of the corpus luteum, prepare the uterus for the reception of the ovum. Finally Loeb has opened a wide field, by discovering a delicate physiological method, by means of which it would seem possible to measure (perhaps even quantitatively) and to test the effect of the active corpus luteum substance.

Attempts to obtain results from the administration of corpus luteum by injection or by the oral route have been made. These experiments were, however, performed with the view of influencing metabolism or of combating the symptoms of the artificial menopause. They were performed more often on the human subject, so that no accurate observations of any local effects could be gathered. Among these studies are the work of Fraenkel, Villemin, Drevet, Lambert, Morley, etc.⁷ Villemin alone attempted a closer pharmacological investigation of the effect of the corpus luteum substance injected intravenously.⁸ He found that these injections produced marked circulatory depressive symptoms, and, in large doses, rapid death preceded by convulsions. He therefore concluded that corpus luteum contained two active principles, the one vasodilator in action, the other convulsant and toxic.

SCOPE OF THE EXPERIMENTS

In the preliminary work undertaken to devise a technic for the study of the effect of corpus luteum on metabolism, as suggested to me by Professor MacCallum, I was impressed by the lack of data relating to the local effect of corpus luteum on the uterus, and by the extreme divergence of opinion as to the gross pharmacological actions of this gland. The study of the effects on metabolism were, therefore, abandoned and the work, to be detailed below, was performed with the object of discovering:

I. The gross pharmacological effects of the intravenous injection of extracts of corpus luteum.

II. If corpus luteum injection can replace the normal secretion of the gland (after removal of ovaries) sufficiently (1) to produce Loeb deciduomata; (2) to bring about estrual changes; (3) to permit the con-

7. Drevet: Effets thérapeutiques du corps jaune, etc., Thèse, Paris, 1907, Steinheil. Lambert: Compt. rend. Soc. d. biol., 1907, lxii, 18. Morley: Detroit, Med. Jour., August, 1909.

8. Lambert reports that fresh corpus luteum extract circulating through the heart of the frog causes arrest in diastole.

tinuation of pregnancy: (4) to maintain the normal condition of the uterus (i. e., avert the atrophy of castration).

III. The effect of corpus luteum injection on the hypophysis of castrated animals.

I. THE PHARMACOLOGICAL EFFECTS OF INTRAVENOUS INJECTION OF CORPUS LUTEUM EXTRACT

The gross pharmacological effects necessarily suggested themselves as the first step in developing a feasible technic. The corpus luteum is incontrovertibly a gland of internal secretion, if it possesses any function. Its secretion, therefore, is normally distributed by means of the blood vessels, though possibly absorption might be effected through lymphatic (subcutaneous) administration, as with adrenalin, or by assimilation through the gastro-intestinal tract, similar to that obtained with thyroid substance. In view of the assertions of Villemin, it became imperative to confirm or disprove his statements.

Experiments.—In all experiments ether was administered for all operative procedures. Hypodermic injections, however, except in dogs, were given without anesthesia.

Preparation of Corpus Luteum Extract.—Dog's corpus luteum substance was obtained by shelling out the corpora from the ovary. The glands were passed through a small meat-chopper, carefully ground in a mortar with sand, and macerated at 0° C. for three hours. The extract was then freed of all coarse particles by rapid centrifugalization for from ten to twelve minutes, and used in the dilutions specified below.

Injections were made into the exposed femoral vein, care being taken to inject at an approximately uniform rate in every instance. The animal's temperature was taken before beginning the anesthesia, during the course of anesthesia, and fifteen minutes after injection.⁹ The pulse-rate and respiration were also noted at similar intervals. Except in the first experiment, all dogs used were females.

I-A. INJECTIONS INTO DOGS OF DOG'S CORPUS LUTEUM EXTRACT

Experiment 1.—Dog, weighing 11¼ pounds; 3 gm. of substance (from 20 ovaries) in dilution of 1 to 6 (9/10 normal salt solution) injected in two equal doses at ten-minute intervals. No circulatory effect, depression of temperature of 3° (ether?). Remained permanently well.

Experiment 2.—Bitch, weighing 9 pounds; 11 gm. of substance in dilution of 1 to 2 injected in two doses (¾ and ¼ of whole). No effect, depression of temperature 4°. Remained permanently well.

9. As no change except a gradual fall of temperature, apparently due to the administration of ether, and proportional to the length of the narcosis, was noted, the temperature is not recorded in the later protocols. Villemin reports a rise after injection.

On account of the difficulty of obtaining material, the succeeding experiments were performed with sheep's corpus luteum, prepared as above.

II-A. INJECTIONS INTO DOGS OF SHEEP'S CORPUS LUTEUM EXTRACT

Experiment 3.—Bitch, weighing $8\frac{3}{4}$ pounds; $7\frac{1}{2}$ gm. substance in dilution of 1 to 2. Death immediate without convulsion; heart continued to beat for several minutes. Autopsy: The right heart was found completely blocked by solid thrombi.

Experiment 4.—Bitch, weighing $9\frac{5}{8}$ pounds. Preliminary injection of 15 gm. of extract from the ovary, deprived of its corpus luteum; dilution 1 to 2. (This extract, made from the more solid portions of the ovary, was found to contain less albuminous substances (boiling) and fewer particles (microscope). No effect except marked, but transitory increase in the respiratory rate. After fifteen minutes interval, injection of 7.5 gm. of the same extract as used in Experiment 3. No effect. Killed with ether after twenty minutes. Autopsy: Numerous hemorrhagic spots found in both lungs. Histological examination showed that the hemorrhagic areas were in the course of distribution of the pulmonary artery. No cell thrombi were found. This was explained by examination of the extract in which no formed cellular elements remained after centrifugalization.

Experiment 5.—Bitch, weighing $10\frac{3}{8}$ pounds; 25 c.c. of fresh dog serum was added to 25 gm. of substance in dilution of 1 to 1, and after fifteen minutes injected in two doses of 20 c.c. and 10 c.c. (corresponding to 20 gm. of substance in dilution of 1 to 2). After the first injection, respiration was very rapid and shallow for six minutes; the second injection, fifteen minutes later, produced no effect. Killed with ether twenty minutes later. Autopsy: Fresh hemorrhagic areas in lungs.

Experiment 6.—Bitch, weighing 10 pounds; 32 gm. substance, dilution 1 to 1. This extract was preserved at 0° for twenty hours instead of three hours. Injection of 8 c.c. produced stoppage of respiration for two minutes; heart slow and feeble; after five minutes a convulsion, then slow recovery. Ten minutes after the first injection, 20 c.c. more were given, heart action very feeble, irregular and slow. Ten minutes later 5 c.c. were given. Gradual recovery. Killed with ether after fifteen minutes. Autopsy: Lungs normal; firm clot almost completely blocking the auriculoventricular opening and extending into the inferior vena cava.

In order to exclude operative intervention rabbits were used for the succeeding experiments. Injections were made into the ear vein. The extracts were from twelve to twenty-four hours old. In the hirudin experiments to be detailed, equal parts of the same extract were employed in hirudinized and control animals. The rabbits weighed from 1,500 to 2,000 gm. and all were females.

II-B. INJECTION INTO RABBITS OF SHEEP'S CORPUS LUTEUM EXTRACT

Experiment 7.—Injection of 6 c.c. of extract 1 to 1; immediate death in convulsion.

Experiment 8.—Preliminary injection intravenously of 0.5 gm. of hirudin in salt solution. After two minutes 6 c.c. of extract 1 to 1. No effect, remained permanently well.

Experiment 9.—Repetition of Experiment 8 in a second animal. Same result.

Experiment 10.—Injection of 2 c.c. extract 1 to 1; no effect. Same extract twenty-four hours later, 2 c.c. Immediate death with convulsion.

Experiment 11.—Injection of 1 c.c. 1 to 1; death in five minutes.

Experiment 12.—Injection of 1 c.c. 1 to 1; convulsions; died in ten minutes.

Experiment 13.—Injection of 6 c.c. of ovarian extract 1 to 1. After fifteen minutes slight opisthotonos, then permanent recovery.

Epicritical Remarks.—The results obtained in the above thirteen experiments conclusively prove that Villemain's deductions are entirely erroneous. The two dogs in whom an homologous extract was used, suffered no ill effect.¹⁰

The four dogs injected with heterologous (sheep's) extract, reacted in different ways; these differences depending on controllable factors. In Dog 3 it was found that approximately 0.75 to 1 c.c. of extract to the pound of body weight produced immediate death. Autopsy showed that death was due to right heart thrombi.

Dog 4 received a preliminary injection of ovarian extract, which prepared from less glandular elements, produced a minor degree of thrombosis, and temporarily reduced the coagulability of the blood.

The succeeding dose of corpus luteum extract, which was sufficient to kill Dog 3 was, therefore, borne without ill effect. Autopsy in this case demonstrated that, as the coagula were slower in formation, and less in degree, the thrombosis did not take place in the heart, but in the branches of the pulmonary artery.

A similar protective action to the lethal dose was obtained in a different way (Dog 5) by partly neutralizing or inhibiting the coagulant effect of the extract by means of addition of fresh dog's serum *in vitro* before injection. Autopsy again showed lesions in the lungs.

In Dog 6 it was hoped to weaken the extract by allowing it to age (twenty hours instead of three hours). The first injection of 8 c.c. was, however, perilously close to the fatal dose. Great cardiac weakness and convulsions supervened, but the dog nevertheless recovered. The dog then bore 25 c.c. in addition, with but slight effects. The first dose had reduced the coagulability to such a degree that double the usual fatal amount was borne without difficulty. Autopsy made evident that thrombi had almost completely blocked the tricuspid opening and the cava, but, through some favorable accidental mechanical factors, the circulation was yet able to continue.

10. In Experiment 1, the great dilution of 1 to 6 accounts for the negative result. In Experiment 2 transient acceleration of respiration was noted. The dog was allowed to live in order to determine whether remoter effects would develop. Had the dog been killed, pulmonary emboli, doubtless, would have been found.

In the rabbits it was found that from 1 to at most 2 c.c. was the fatal dose (in Rabbit 10, although 2 c.c. failed to kill on the first day, yet 2 c.c. of the same extract, twenty-four hours later, produced immediate death).

By giving a preceding injection of hirudin (purified leech extract), which completely inhibits the coagulability of the blood for several hours, three times the fatal dose was found to produce no ill result. In all the rabbits that died, solid thrombi filled the right auricle and ventricle.

Deductions Based on the Experimental Findings.—The results obtained by Villemain are, therefore, to be interpreted as due to intravascular thrombosis and not to either a vasodilator or general toxic action. Neither flushing of the peripheral parts nor increased peristalsis, as described by this author, were noted in my series in any instance.

A word of caution to investigators who employ the intravascular route for administration of tissue extracts may be permitted. Naunyn and Rauschenbach¹¹ have shown that coagulating substances can be obtained from any and all cells. Such substances as thrombokinase or those acting similarly are closely bound to the protoplasm. Consequently, when more minute and complete subdivision of cells is obtained, more of these thrombogenic substances are set free. On the other hand, larger aggregations of cells produce thrombosis through their action as foreign bodies, rather than through the ferments set free. Veit and his pupils largely based their placental toxin theory on the convulsion produced by intravascular injections of placental extracts. It has required the combined efforts of many investigators—Lichtenstein, Mathes, Schenk, etc.,¹² employing the methods used in my investigation, to undermine and, let us hope, to lay permanently at rest these misleading conclusions.

The results of intravascular injections of corpus luteum extract, in massive doses, produce intravascular coagulation without any well-marked pharmacological effect.

II. CAN CORPUS LUTEUM INJECTIONS REPLACE THE NORMAL SECRETION OF THE GLAND?

The preliminary investigation demonstrated that intravenous administration of corpus luteum could not be performed with safety. Throughout the rest of the experiments subcutaneous injection, or administration by mouth, or a combination of the two were employed.

11. Naunyn: Arch. f. exper. Path., 1873, i, 1. Rauschenbach: Ueber die Wechselwirkung zwischen Protoplasma und Blutplasma, Inauguration Diss., Dorpat, 1883. Loeb, L.: Med. News, 1905, lxxxvi, 577.

12. Lichtenstein: Zentralbl. f. Gynäk., 1909, xxxiii, 1313; Arch. f. Gynäk., 1908, lxxxvi. Mathes: Zentralbl. f. Gynäk., 1908, xxxii, 1548. Schenk: Zentralbl. f. Gynäk., 1909, xxxiii, 1500.

As Loeb has definitely shown that in the guinea-pig and rabbit the uterine mucosa reacts in a well-defined and easily recognizable manner to the chemical action of the corpus luteum, these animals were chosen for my experiments. The work of Bouin and Ancel did not appear until after most of the experiments had been concluded. Nevertheless, all the sections were reexamined with a view of determining whether or not the histological changes, described by these authors, were present.

As the technic throughout was identical, and variations were limited to the interval between double oöphorectomy and incision of the uterus, and to the quantities of corpus luteum extract injected and the route of administration, I am able to summarize the results most briefly.

METHODS EMPLOYED

Extract.—All the extracts were obtained from the fresh corpora lutea of sows. The ovaries were dipped for an instant into 4 per cent. liquor formaldehydi and then rapidly washed in two changes of sterile water to eliminate surface bacteria. The remaining steps were identical with those described in connection with the previous series, except that asepsis was more rigidly observed. The amount of normal salt solution used was 1 to 1 by weight. Fresh extracts were prepared every seventy-two hours, so that the extracts actually injected were twelve, twenty-four, thirty-six, forty-eight, sixty and seventy-two hours old; then the series was begun again.

Method of Administration.—For hypodermic use extracts were centrifuged for five minutes; for mouth administration the unclarified extracts were used. The site of injection preferably was the back. In a few instances some induration resulted; in one case an abscess developed. In the great majority of experiments the fluid was absorbed rapidly and completely. The extract, when injected subcutaneously, was diluted with an equal quantity of salt solution. Quantities of extracts, as given in the protocols, are based on the amount of solid substance from which the extract was prepared.

Operative Procedure.—The technic employed was that of Loeb. Dr. Loeb very kindly demonstrated his methods to me and permitted me to examine his specimens. In addition, he offered many practical suggestions and cautions which proved invaluable. The ovaries were removed through the lumbar route. This operation can be performed without shock or interference with the uterus, in from eight to fifteen minutes. Incisions of the uterus were made through a median abdominal incision. A part of each horn of the uterus was split along its anterior surface and numerous transverse cuts made in addition.

The rabbits survived these two procedures without trouble. Numerous guinea-pigs, however, were lost. The following is a brief summary of the protocols of the animals which survived sufficiently long to prove of value:

EXPERIMENTS ON RABBITS

Experiment 14.—Double oöphorectomy and incision of uterus at one operation. Injections of 0.5 gm. every twenty-four hours begun on eighth day. Died eight days later (sixteen days after operation). Two masses, respectively, the size of small and large pea at autopsy.

Experiment 15.—Same as 14, except that 1 gm. was given every twenty-four hours. Killed on sixth day (fourteen days after operation). Everted mucosa forming small rosettes.

Experiment 16.—Double oöphorectomy; 2 gm. by mouth twice daily throughout; incision of uterus on fifth day. Killed six days after second operation. Bright red nodular masses.

Experiment 17.—Same as 16, except that 1 gm. was given twice daily and that the animal was killed on the twelfth day after the second operation. The uterine incision showed much everted hypertrophied mucosa.

Experiment 18.—Double oöphorectomy (large corpora lutea); 1 gm. by hypodermic injection, mornings, and 1 gm. by mouth, evenings, throughout. Incision of uterus on the fifth day. Died on eighth day after second operation. Nodules along line of incision.

Experiment 19.—Same as 18, except that 0.5 gm. was given by hypodermic injection and 1 gm. by mouth. Died on tenth day after second operation.

Experiment 20.—Double oöphorectomy (large corpora lutea); incision of uterus on fifth day. Killed nine days after second operation. No injections. Used as control.

EXPERIMENTS ON GUINEA-PIGS

Experiment 21.—Double oöphorectomy and incision of left horn (in right horn three pregnancies of fifteen days); 0.5 gm. by hypodermic injection; killed tenth day. No nodules left horn. Pregnancies nearly absorbed.

Experiment 22.—Double oöphorectomy; 0.5 gm. by hypodermic injection; 1 gm. by mouth. Aborted on fourth day.

Experiment 23.—Double oöphorectomy; 0.5 gm. by hypodermic injection; 1 gm. by mouth. Aborted seventh day.

Experiment 24.—Double oöphorectomy and incision of uterus; 1 gm. hypodermic injection; killed ninth day. No nodules.

Experiment 25.—Double oöphorectomy; 1 gm. by hypodermic injection every day. Incision of uterus sixth day. Died next day.

Experiment 26.—Double oöphorectomy; 1 gm. by hypodermic injection every day. Incision of uterus seventh day. Died next day.

Experiment 27.—Double oöphorectomy; 1 gm. by hypodermic injection for five days. Incision of uterus fifth day; 0.5 gm. by hypodermic injection thereafter. Killed ten days later. No nodules.

Experiment 28.—Double oöphorectomy; 2 gm. by mouth twice daily. Died from anesthetic fifth day.

Experiment 29.—Double oöphorectomy; 0.5 gm. by mouth twice daily. Incision of uterus fifth day. Died four days later. No nodules.

Experiment 30.—Double oöphorectomy; 2 gm. by mouth twice daily throughout. Incision of uterus fifth day. Killed ten days later. No nodules.

Experiment 31.—Double oöphorectomy; 0.5 gm. by hypodermic injection; 2 gm. mouth daily throughout. Incision of uterus fourth day. Killed twelve days later. No nodules.

Experiment 32.—Double oöphorectomy; 0.5 gm. by hypodermic injection, 1 gm. by mouth daily throughout. Incision of uterus seventh day. Died five days later. No nodules.

Experiment 33.—Double oöphorectomy; 0.5 gm. by hypodermic injection; 0.5 gm. by mouth daily throughout. Incision of uterus ninth day. Died two and one-half days later. Numerous small nodules.

Experiment 34.—Double oöphorectomy; incision of uterus eighth day. Killed six days later. Control.

Experiment 35.—Double oöphorectomy; incision of uterus fifth day. Killed seven days later. Control.

Experiment 36.—Double oöphorectomy; incision of uterus fifth day. Killed ten days later. Control.

HISTOLOGICAL EXAMINATIONS

All suspicious nodules in the line of incision, and a portion of the uterus above and below the operation wound were examined. On the average five blocks from each case were cut in serial section and stained with hematoxylin and eosin.

Results in Regard to Deciduomata of Loeb.—Loeb has shown that, in response to appropriate stimuli, deciduomata appear in the guinea-pig and rabbit from two to nine days after the formation of the corpus luteum.

As injections might not correspond to Nature's efforts, variations of time, dosage and method of administration were employed. Experiments 14 to 19 and 24, 27, 29, 30, 31, 32 and 33 can be utilized in this connection.

Although, particularly in the rabbits, and in guinea-pigs 21 and 23, macroscopic nodules were present, deciduomata according to Loeb's histological criteria were uniformly absent.

Results in Regard to the Estrual Cycle.—As previously noted certain changes occur in the surface epithelium of the rabbit's uterus (probably also in that of the guinea-pig) during the interval between each period of rut. Loeb found such changes—amitotic nuclear proliferation—about the fifteenth day, not on the third or fifth day. Bouin and Ancel assert that both macroscopic and microscopic alterations can be observed within forty-eight hours of a non-fecundating coitus (male with vasa deferentia ligated), and that these changes increase progressively until the fourteenth day. They report that the nuclei form columns and that the cytoplasm appears as undivided symplasmatic sheets. Removal of the corpus luteum produces rapid regression at any stage.

Without entering into any discussion of the discrepancies observable between these views, I may state that, in the uteri of neither the rabbits nor the guinea-pigs of my series were any nuclear proliferations noted.

Results in Regard to Continuation of Pregnancy after Castration.—Fraenkel and others have asserted that castration or extirpation of the corpus luteum, early in pregnancy, produced absorption of the ovum. Considerable doubt has been cast on these observations.* Whether the continuation of pregnancy depends on the formation of the maternal

* Loeb and Hunter: (Univ. Penn. Med. Bull., 1908, x, 294) found that removal of the mammary gland frequently produced absorption of the pregnancies or abortion in guinea-pigs.

decidua (as shown by Loeb), and whether this structure is essential for nutritional purposes, I am unable to state. Quite possibly, however, the decidua is merely a protective reaction against the invasive action of the trophoblast.

My experiments (21, 22 and 23) are too few and incomplete to add anything to our knowledge. In Experiment 21 the operative interventions were sufficiently extensive to account for the absorption of the pregnancies. In Experiments 22 and 23 abortion occurred on the fourth and seventh day after operation. The injection of extract certainly did not produce any increased stimulus toward a continuation of pregnancy.

Results in Regard to Castration Atrophy.—It is well known that castration is followed by atrophy of the uterus. The statements regarding the rapidity of this process vary somewhat. Fraenkel says that fourteen days after castration the rabbit's uterus is a narrow, flaccid yellowish band. The muscle layer has atrophied to such an extent that the thickness of the mucous membrane exceeds it. Bouin and Ancel state that the atrophy is rapid.

In order to be in a position to determine this point in my experiments, controls were essential. For comparison I chose Rabbits 18 and 20, the ovaries of both containing well-developed corpora lutea. Guinea-pigs 31 and 36 afforded a similar contrast. The ovaries of Guinea-pigs 27, 30 and 34 contained no corpora lutea. Guinea-pigs 32 and 35 were also of value in this connection. In addition all the other uteri were studied in order to determine whether atrophy was progressive and proportional to the time elapsed since castration, and whether the injections had any recognizable influence upon involution.

The observations extend only over a period from five to seventeen days after oöphorectomy. Both mucosa and muscle layer appeared normal. Possibly the nuclei of the epithelial cells were somewhat narrower than in non-castrated animals, but no distinct histological evidence of atrophy was observed in either the injected or control animals within seventeen days of castration.

III. THE EFFECT OF CORPUS LUTEUM INJECTION ON THE HYPOPHYSIS OF CASTRATED ANIMALS

The interaction and interdependence of the glands of internal secretion has been recognized by clinicians for some time. More recently, experimental investigators have sought to define these relationships and to discover the laws which govern their compensatory action.

For instance, absence of the thyroid gland, as in cretinism, is usually accompanied by genital hypoplasia. Acromegaly, due to tumors of the

hypophysis cerebri, is commonly followed by infantilism of the female generative glands. Adrenal tumor has been known to change the secondary female sexual characteristics into those of the opposite sex (case of Thumin¹³). Such observations can be indefinitely multiplied. They are of more than passing interest, but, beyond calling attention to the marvellous interaction of the organs of internal secretion, throw no light upon the subject.

Even in normal pregnancy certain symptoms, such as coarsening of the face, growth of hair, etc., point to hypophyseal hyperactivity. Erdheim and Stumme,¹⁴ in a thorough anatomical study, have shown that during pregnancy the hypophysis enlarges and undergoes characteristic morphological changes.¹⁵ In order to make these clear, it will be necessary to enumerate briefly the histological constituents of the gland.

The hypophysis is composed of three parts, the posterior or nervous portion, with which we may include the infundibulum, the pars intermedia, or *Epithelialsaum* and the anterior lobe, which is glandular. The nervous portion of the hypophysis does not concern us here. As physiologists have shown, its extracts have a strong pressor (vasoconstrictor) influence, similar to adrenalin, and, according to some, the extract acts as a powerful oxytoxic (Bell¹⁶). The pars intermedia varies in different species, and is of interest largely because within it may be found glandular spaces, which resemble thyroid acini, and contain colloid within their lumen.

The anterior lobe chiefly engages our attention. It is composed of closely packed groups of cells, so arranged that nearly every individual cell is in direct contact with the walls of one or more capillaries. Three varieties of cells have been described. Quite probably, all three express merely different stages of function or metabolic activity. This classification is based upon the staining properties of the cells. Acidophil (eosinophil) cells under ordinary conditions predominate in number; basophil (staining with hematoxylin, etc.) are numerically second, and a smaller number of chromophobe cells (whose protoplasm is indifferent to both acid and basic dyes) are also present. For details, the reader is referred to Erdheim and Stumme, and to an excellent morphological study of the hypophysis in mammals, by Trautmann.¹⁷ A complete review of the

13. Thumin: Berl. klin. Wehnschr., 1909, xlv, 103.

14. Erdheim and Stumme: Beitr. z. path. Anat. u. z. allg. Path., 1910, xlv, 1.

15. Comte: Beitr. z. path. Anat. u. z. allg. Path., 1898, xxiii, 90. Launois, P. E., and Mulon, P.: Etude sur l'hypophyse humaine à la fin de gestation, Compt. rend. de l'Assn. d'anat., Nancy, 1903, v, 124.

16. Bell: Brit. Med. Jour., 1909, ii, 1609.

17. Trautmann: Arch. f. mikr. Anat., 1909, lxxiv, 311.

literature will be found in Paulesco's monograph.¹⁸ Erdheim and Stumme have shown that in the course of pregnancy the basophilic cells increase enormously in number, most probably at the expense of the eosinophilic cells. It is of interest and importance to determine in response to what stimuli these changes occur.

In pregnancy we have two factors to consider in this connection. The one is the fetus and placenta, the other the persistent corpus luteum (corpus luteum of pregnancy). According to Claypon and Starling¹⁹ extracts of the fetus—not of the placenta or of the uterus—produce hypertrophy of the mammary gland. Withdrawal of this influence is followed by the secretion of milk from the activated gland. This observation, solitary and unconfirmed, permits of no generalization, but draws attention to the fact that the fetal influence might also exert some power on other glandular organs, as, for instance, on the hypophysis.

In regard to the corpus luteum, numerous experiments have been made, in which the corpus luteum secretion has been completely withdrawn by means of castration.

All investigators report hypertrophy of the pituitary gland in castrated animals of both sexes. They also describe changes in the numerical proportion between the three types of cells, etc.

Of the various observations reported, we may accept as proved that the hypophysis is found increased in weight in castrated animals. This enlargement must be considered a compensatory hypertrophy. Even in this connection, however, possibilities of error arise, for the hypophysis under normal conditions gains in weight progressively with the increased age of the animal (Trautmann).

When we turn to the morphological changes, the possibilities for error, and for subjective interpretation of the findings, are greatly increased. Even in the thinnest sections, a bewildering maze of cells is encountered, and sections cut in different planes will give differing pictures. Therefore, before we can accept reports of variations in the numerical proportion of cells, these variations must be so marked as to preclude misinterpretation.

I will spare the reader the task of wading through even the sifted and epitomized mass of literature which has been written on this subject. Not one of the interpretations based on variations in morphology—except that in pregnancy—appears convincing to me.^{18, 20}

18. Paulesco, N. C.: *L'hypophyse du cerveau*, I Physiologie, Paris, 1908.

19. Lane-Claypon and Starling: *Proc. Royal Soc.*, 1906, lxxviii, B, 505; *Starling*: *Lancet*, London, 1905, iv, 579.

20. Delille: *L'hypophyse et la médication hypophysaire*, Paris, 1909.

MATERIAL AND METHOD

The corpus luteum injections, previously detailed, afforded me the opportunity of studying their effect on the hypophysis as well as on the uterus. At Dr. MacCallum's suggestion I have done so.

The protocols above recorded sufficiently explain the nature of the experiments. Corpus luteum extract was given to castrated animals for periods varying in length up to fifteen days. The hypophysis of normal pregnant and non-pregnant guinea-pigs and rabbits, and of castrated animals not injected with extract, were used for controls. As the hypophyses were too small to permit of subdivision, fixation of each specimen in but one fluid was possible. Alcohol and formaldehyd solution were used. This choice proved unfortunate, as several of the special stains recommended failed to give good results. A good nuclear hematoxylin (Delafield) with eosin as counterstain gave satisfactory pictures in which the three cardinal varieties of cells could be clearly distinguished.

The hypophyses of twenty-seven rabbits and guinea-pigs were examined. Histological examination showed no recognizable difference between the hypophyses of the castrated animals, injected with corpus luteum extract, and those of the controls.

OTHER OBSERVATIONS

A few thyroid glands were also examined. The structure of the thyroid is too well known to require special description. Here also no characteristic changes were noted either in the epithelium or in the amount or distribution of the colloid secretion.

The suprarenal glands likewise appeared normal in every respect. Examination of the kidneys showed no degenerative or other changes. This applied both to those animals which had received subcutaneous doses of extract, as well as to those to whom it was given by mouth. Therefore, we may conclude that the extract does not irritate the kidneys.

Although numerous animals died during the course of the experiments, their death cannot, in most instances, be directly ascribed to any specific toxic action of the corpus luteum extract. The administration, furthermore, of such enormous doses as were given (for instance 2 gm. of extract given twice daily to a guinea-pig of 500 gm. weight would correspond to nearly 0.5 kilo of extract daily to a woman weighing 60 kilos) are far in excess of any amount which might be employed in actual therapy.

SUMMARY OF EXPERIMENTAL RESULTS

The results of this investigation can be summed up most briefly:

1. Corpus luteum extract, injected intravenously in sufficient concentration, proves rapidly fatal in consequence of intravascular thrombosis.
2. Corpus luteum extract of a heterologous species, given subcutaneously, by mouth, or by a combination of these routes, does not replace the normal action of this gland of internal secretion. The injections do not

suffice to "sensitize" the uterus and enable it to produce Loeb's deciduomata and do not bring about such epithelial changes as are noted after follicular rupture.

3. Corpus luteum extract injections call forth no recognizable reaction in the hypophysis.

OVOTHERAPY VIEWED IN CONNECTION WITH EXPERIMENTAL RESULTS

Negative results in any investigation always prove disappointing. Nevertheless, in this instance, they possess a practical value. Ovotherapy has for some years been widely employed in an empirical way, either in the form of ovarian extract or of the more specific (?) lutein substance. The results obtained have shown great variations, seemingly depending about equally on the subjective impressions of the physician and of the patient.

That castration exercises an influence on the organism is beyond dispute. The nature of these alterations, however, is by no means constant. In young animals the sexual characteristics, particularly the secondary characteristics, may assume a neutral type, and the normal development of the organs of sex and of the skeleton is usually interfered with. In the adult human being organic alterations, except in the genital apparatus, are minimal, and many of the symptoms complained of are largely psychological, perhaps due to a great extent to a popularly ingrained superstition, amounting to a "phobia."

For many years a profound influence on the general metabolism has been supposed to follow castration. Loewy and Richter,²¹ investigating the cause of obesity in castrates, reported diminution of oxidation in both male and female dogs; and a return to above the normal, in both sexes, in response to administration of ovarian (or testicular) substance in the food. Their examinations were limited to the oxygen consumption, and carbon dioxid excretion. The newest, and I may say most thorough work, is that of McCrudden,²² who did not find the commonly accepted alterations in metabolism, which supposedly follow castration.

Therefore, not only must we be prepared to revise our views on the after-effect of castration, but also, for the present, to assume a judicial attitude toward the contradictory statements, relating to the effects of ovotherapy.

21. Loewy and Richter: Berl. klin. Wchnschr., 1899, xxxvi, 1095.

22. McCrudden: Jour. Biol. Chem., 1910, vii, 185.

In castrated women, return of the menstrual function, as observed after implantation of ovarian tissue, will afford an unmistakable index of the efficacy of organotherapy; in animals, Loeb's test can be applied.

The symptom most frequently relieved by oötherapy is the so-called flush (sweats, dizziness, etc.) of the natural and artificial menopause. These vasomotor disturbances, most often noted in neurotic women, may persist for a shorter or longer period; may disappear in response to appropriate or inappropriate treatment, and are, therefore, entirely unsuitable as a reliable index for judging the efficacy or potency of any drug.²³

In the further study of the corpus luteum our efforts should be directed toward exact experimental proof rather than toward empirical generalization. Possibly, by different methods, an active substance may yet be obtained; probably, however, Loeb is correct in saying that "it is very likely that the body fluids are different in the different individuals of the same species." There hinges the difficulty, and the obstacles may prove insurmountable, when we are forced to employ substances derived from a different species, although this generalization finds at least one exception, the case of the thyroid gland.

Further experiments along these lines will be undertaken in the near future. The subject is of too great importance to be dismissed before every effort has been exhausted.

I desire to thank Professor MacCallum for the numerous suggestions, already acknowledged in the body of this article, and for extending the privileges of his laboratory. I am under deep obligations to Dr. Leo Loeb of Philadelphia for his kindness in acquainting me with the technic he has developed and for numerous other courtesies. Dr. Howard Lindemann assisted me throughout the experimental work, and my obligations also include several of the workers in the laboratory, who have materially aided me in many ways.

983 Park Avenue.

23. It would prove of interest, in this connection, to make a careful study of the variations in blood-pressure before and after castration, and the effect (?) of oötherapy under these conditions.