in part at least, by the decline of the corpus luteum.

In an earlier note to SCIENCE (84: 161, 1936) we reported that the injection of an estrogen during the latter part of pseudopregnancy causes the corpora to persist in a functional manner. In view of the supposition that the corpora become non-functional shortly before parturition, we have carried out a series of experiments designed to test the effect of an estrogen given during the last few days of pregnancy, expecting, of course, that if its effect on the corpora be similar to that obtained during pseudopregnancy, the corpora of pregnancy would be maintained and parturition would be delayed. The results have shown this to be so. Daily injections of estradiol monobenzoate (Progynon-B) in doses varying from 50 to 500 i.u. per kilo beginning at any time from the 27th to the 31st day of pregnancy postponed delivery in most cases. In only one of nineteen animals did normal delivery of living foetuses occur at the usual time (32 days), and this was one in which injections were started on the 31st day. In fourteen of these animals parturition was delayed longer than 35 days. In the experiments thus far carried out the foetuses have been dead whether delivered or obtained by operation or autopsy. In no case as yet have we been able to obtain post-mature foetuses in animals in which parturition has been delayed with estrogenic hormone. They are killed within two days and are retained in utero. Animals killed on the 37th, 45th and 52nd days have shown macerated foetuses in utero and moderate-sized corpora lutea in the ovaries which microscopically looked very much like normal corpora. It will be remembered that the corpora of pregnancy look quite degenerate by the 35th day, *i.e.,* three days after normal parturition. It is also of interest that animals in which parturition has been prevented in this manner may show extreme necrosis of the uterine muscle. For instance, the animal killed on the 52nd day showed almost complete necrosis of the muscle overlying the foetuses. Delivery obviously would be impossible in such a case, but this can scarcely be considered the cause of the original delay in parturition, since animals in which the uteri were examined sooner showed less necrosis, and an animal subjected to laparotomy on the 32nd day showed no evidence of necrosis although the foetuses were already dead.

Control experiments have been made which indicate that the ovaries must be present if delay in parturition is to be obtained by the injection of estrogenic hormone. Four animals were castrated on the 27th day and injections of estradiol monobenzoate started the same day. Delivery occurred spontaneously on the 29th and 30th days, and living foetuses were present in all the litters. In three other animals the procedure was modified somewhat. Injections were started on the 29th and 30th days and the ovaries were removed three to five days later. All three animals delivered dead, macerated foetuses within three days after castration.

Our results show that the hormonic conditions present at the end of pregnancy may be altered in much the same manner as those at the end of pseudopregnancy. In each case the regression of the corpora is prevented by increasing the estrogen level. This indicates that there is normally no rise in the estrogen level at the end of pregnancy, as suggested by some authors.

The results which we have obtained call for an interpretation of the effect of estrogenic hormone in pregnancy in the rabbit different from that usually advanced. There are numerous references in the literature indicating that estrogens induce abortion. It has been shown that in the last third of pregnancy in the rabbit injection for only a day or two of estrogen results in premature delivery of dead foetuses within three or four days. We have obtained similar results by giving the hormone in single doses or for a limited time, but when repeated doses are given, the foetuses are retained. The explanation usually given for the abortive action of estrogen in pregnancy is that it increases the irritability of the uterine muscle to such an extent that expulsion of the foetuses takes place. Our findings suggest a different explanation. Under normal circumstances the corpora lutea are caused to remain functional during the latter half of pregnancy by some hormone elaborated by the placentas or foetuses (the presence of such a hormone has been demonstrated) but when an estrogen is given for a limited time, the placentas and foetuses are so damaged that they are no longer capable of maintaining the corpora. When the inhibiting effect of the corpus luteum hormone is removed, premature delivery of dead foetuses occurs, a result similar to that which follows castration, excepting that in the latter case the foetuses are usually living. When repeated, daily injections of an estrogen are given, as in our experiments, the placentas are injured in the same way and the foetuses die, but the corpora are maintained by the estrogen and as a result the foetuses are retained in utero.

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LOCALIZATION OF CALCIUM IN PARAMECIUM CAUDATUM

IN a series of preliminary microincineration experiments (unpublished) on the liver and brain of

young and old rats, the writer observed an increase with age in the quantity of cortical ash in the individual cells. Scott,[1] using the microincineration technique, observed a concentration of ash at both the nuclear and cell membranes. The following experiments were conducted to determine the nature of the cortical ash with particular reference to calcium.

In using the microincineration technique as a means of localizing the calcium in metazoan cells the question might arise as to whether any calcium at the cell membrane would be intercellular or intracellular. In order to avoid this possibility, the protozoan *Paramecium caudatum* was used as the experimental organism. Paramecium also has the advantage of size; it is large and comparatively easy to work with.

Procedure

The microincineration technique as developed by Policard[2] and Scott is essentially that which was used in the following experiments. The material was fixed in Bouin's Fluid (minus the glacial acetic acid); the absolute alcohol and formalin fixative as recommended by Scott was also used. Both dioxan and alcohol dehydration were employed with no apparent variation in the results. The material was imbedded in paraffin in the usual manner and sectioned at 8 micra. The sections were mounted on clean slides and incinerated in an electric furnace at approximately 600° C., for 30 minutes. The material was then studied by means of the dark-field microscope.

Two specific colorimetric tests for calcium were used; sodium alizarin sulfonate, which produces a red precipitate in the presence of calcium,[3] and the gallo-formic reaction, which produces a blue precipitate specific for calcium.[4] The reagent as used by the writer consisted of a solution of concentrated gallic acid and 40 per cent. formaldehyde (1–1) rendered alkaline (pH 9.0) by the addition of concentrated NH_4OH. The gypsum reaction was also used to a small extent. This consists of dissolving the salt in a microdrop of $0.1N \cdot HCl$ and precipitating the typical $CaSO_4 \cdot 2H_2O$ crystal by adding a microdrop of $0.1H \cdot H_2SO_4$ (Scott).

Results

Random samples of paramecia consistently showed a heavy concentration of salts in the cortex of the cells. The cortical layer of ash was uniform in thickness throughout. A faint pink color was observed, which was imparted to the ash by the presence of iron. Occasional granules of ash were observed in the cell which were probably the inorganic remains of food granules, etc. The thickness of the outer ring of cytoplasmic ash varied considerably. While no actual measurements were done on the thickness of the layer, two distinct extremes were observed, a thin relatively smooth layer of ash on the one hand, and a heavy, granular layer on the other.

Sections treated with sodium alizarin sulfonate showed a distinct change in the color of the cortical layer of ash. The original light pink color gave way to the purplish red, which is characteristic of the alizarin reaction. However, since there does exist a general similarity between the original pink and the final red, the gallo-formic reaction was employed as a check. A bluish-violet color in the cortical ring confirmed the alizarin test for calcium. As previously stated, the gypsum reaction was employed to a small extent. The writer found that the colorimetric methods were sufficiently more positive to warrant their exclusive use. Actually, it was hardly necessary to test for the presence of calcium in the cortical ring of ash. Since almost all the inorganic salts were concentrated in that region of the cell, and since calcium is known to be present in the cell, it would follow that the calcium must be in the cortex. The variation in the thickness of the cortical ring of ash prompted the writer to repeat the experiment using organisms of known ages. (This investigation is being conducted at the present time.)

Summary

By means of the microincineration technique and two specific colorimetric tests for calcium, the writer has demonstrated that most of the cytoplasmic salts are concentrated in the cortex of the cell and that calcium is a constituent of the cortical ash.

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[1] Scott, *Am. Jour. Anat.*, 53: 243, 1933.
[2] Policard, *Protoplasma*, 7: 464, 1929.
[3] Pollack, *Jour. Gen. Physiol.*, 11: 539, 1928.
[4] Cretin, *Bull. Am. Chem. Soc.*, 33: 1551, 1923; *Bull. Hist. Appl.*, 1: 125, 1924.

THE PRESENCE OF A TYPE- AND SPECIES-SPECIFIC CONJUGATED POLYSACCHARIDE IN TYPE I PNEUMOCOCCUS

THE findings of numerous investigators have shown that each pneumococcal type elaborates a type- and a species-specific polysaccharide. They have been isolated individually and characterized chemically. Since heat, acid and alkali have been employed in their isolation, and since autolysates or highly autolyzed organisms have been used as the source of material, it has appeared probable that the polysaccharides thus isolated were fragments or modified parts of a higher complex which might be present in the young and actively growing Gram-positive organisms.