5791

Embryonic Rat Tissue Survival.

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Observations upon developing living mammalian foetuses have been restricted mainly to their movements.[1, 2] Since earlier stages of development offer definite information concerning the mechanics of mammalian morphogenesis, the problem of embryonic survival under varied conditions is of utmost importance. With this in mind the following experiments have been performed.

The rat embryos (8 and 9 days of gestation age) were carefully dissected free from the uterus and the *decidua capsularis* removed. The embryonic membranes which are just forming at the latter stage were left intact. The embryos were placed in Ringer's, Locke's, and physiological saline solutions and their morphological reactions noted. They were removed from the solutions at various times and fixed for histological study.

While the survival time varied considerably, no signs of histolysis were apparent after 6 hours in any of the above solutions at 37.5°C. When kept at 24°C. in the same solutions there was no sign of disintegration 12 hours after removal. Twenty embryos were preserved at 6 and 12 hour periods. The 12-hour embryos at body temperature showed considerable disintegration. The embryo retains its external form up to 72 hours at 24°C. but its cellular structure becomes atypical and shows characteristic degenerative changes. No increase in cellular differentiation occurs.

To determine whether embryos would develop in the mother outside of the abdominal cavity, a subcutaneous pocket was made in the body wall in which the horn of the uterus was placed with its contained embryos (8-9 days of age). The vascular connections were left intact and passed through incisions in the body wall. At the time of operation the embryos were normally imbedded in the uterine mucosa and the specimens removed for study were of 8 days' gestation age. Twelve such cases were permitted to carry their young to term. All except one had a normal birth and the animals so born were viable. Three other cases were preserved at stages intermediate between the stage of operation and full term. All the fetuses (14) were perfectly normal in development.

[1] Swenson, E. A., *Anat. Rec.*, 1925, **30**.

[2] Hooker, D., and Nicholas, J. S., *J. Comp. Neur.*, 1930, **50**.

In the rat the period of egg segmentation occurs in the Fallopian tube. If the tube is separated from the uterus on the third day after fertilization and the uterine lumina are ligated, the segmenting eggs fall into the abdominal cavity where they may either degenerate or continue their development. In 35 experiments, 5 positive results were obtained. Since this means 5 individuals in a total calculated as 250, positive results can be expected in only about 2%. These develop to term, have no decidua, but have a placental mechanism which works through vessels imbedded in the mesenteries.

Attempts to secure development of embryos by transplanting to various locations within the abdomen were unsuccessful. For this reason an investigation of transplantation sites outside the abdomen were made. Eight and 9-day rat embryos were transplanted to the outside of the body wall, to the fascia of the femoral region, and to various subcutaneous areas. In a few cases positive results were indicated and the embryonic transplant was vascularized

The region of the mammary gland forms the best site so far secured. The response to the transplant is shown by hyperemia in the region of the transplant. Since this response might be assumed to be due to the mechanical irritation superimposed upon the hyperemia normal to the mammary gland during the later stages of pregnancy, similar transplantations were made in young male rats, 80 gm. in weight. Here the possible influence of ovarian secretions as well as cyclic mammary hyperemia was eliminated. Eighty percent of the grafts so made were recovered (40 cases, 4 grafts each) from 5 to 10 days subsequent to transplantation. Serial sections through the grafts show differentiating embryonic tissues, definitely recognizable as tissue developed from the embryonic transplant.

The following tissues have so far been identified, differentiated gut including circular and longitudinal musculature along with the intestinal epithelium, cartilage, embryonic red blood cells, striated muscle, nervous tissue and placental cells. The grafts resemble those described by Murphy[3] and Willier[4] after transplanting tissues upon the chorioallantoic membrane of the chick.

The results so far obtained justify the following conclusions: (1) Rat embryos will withstand the handling necessary for experiment. (2) They will survive immersion in salt solutions for long periods without histolysis. (3) Extrauterine pregnancy may be brought about in a limited number of cases by cutting the uterine

[3] Murphy, J. B., *J. Exp. Med.*, 1913, **17.**

[4] Willier, B. H., *Am. J. Anat.*, 1924, **33.**

horn after fertilization and permitting the segmenting blastulae to fall into the abdominal cavity. (4) Differentiation and growth occur regularly in embryos after transposing the uterus from the abdominal cavity to a pocket under the skin outside the abdominal cavity. (5) When embryos (without decidua) are transplanted to the mammary gland, either of males or females, differentiation of tissues takes place. The tissues are not organized to form a whole embryo.

5792

Physiology of the Metabolism of the Purine Nucleosides.

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Upon feeding the purine nucleosides, guanosine and adenosine, to dogs maintained on a synthetic diet, we find amounts of 2 to 3 gm. almost completely metabolized.

In case of guanosine about one-half of the nitrogen is metabolized to allantoin. One-third of the nitrogen is accounted for by an increased output of urinary urea. This is more than could arise from the amino group of the guanosine alone.

In the case of adenosine, we find the same increase in urinary allantoin, but practically no increase in urinary urea or ammonia.

Our results seem to indicate that in the dog the end product of purine metabolism is not allantoin alone; part of the allantoin seems to be further broken down to urea.

The experiments were carried out on 4 dogs. For a description of the experimental technique we refer to our previous publications.[1]

Experiments dealing with the metabolism of the purine bases, guanine and adenine, are in progress.

[1] Cerecedo, L. R., *J. Biol. Chem.*, 1931, **93**, 269.