

When 50 per cent suspension of washed guinea pig corpuscles was injected into rat foetuses, there was a response, on the part of the foetus as shown by tests for hemolysins and agglutinins in the foetal blood. The maternal blood showed, however, no increase in hemolysin either in the general circulation, or in the uterine vein blood. Instead, a substance was found which retarded and prevented the hemolytic reaction. This substance measured 25 units in the uterine vein, and as high as 1 to 2 units in the general circulation. The unit of measurement consisted of the prevention of the hemolytic process of 0.01 cc. of hemolysin (titer 500) by 0.1 cc. of serum.

When females were injected to produce hemolysins before conception, the hemolysin was not passed to a subsequent litter in demonstrable quantity, although the titer of the maternal serum was as high as 300 or 400.

Natural hemolysins, when found in the maternal serum, could not be demonstrated in the young.

The placenta of the white rat acts as a barrier and not as a semi-permeable membrane to hemolysins prepared in the rat against guinea pig corpuscles. In some way the characteristic reaction of the hemolytic body is changed or else an antibody or neutralizing substance is formed, probably by the placenta. There is, of course, a possibility of this substance being formed in both the mother and the foetus but if such is the case, it is probably in much smaller quantity than that which is formed by the placenta.

The placenta of the white rat, therefore, restricts the passage either from mother to foetus or foetus to mother of natural hemolysin and of both actively and passively acquired white rat-guinea pig hemolysin.

¹ Nicholas, J. S., *Anat. Rec.*, 1925, xxxi, 385-394.

² Nicholas, J. S., *PROC. SOC. EXP. BIOL. AND MED.*, 1926, xxiii, 436-439.

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Hormone Content of the Placenta and Chorionic Membranes.

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Earlier investigators have studied the hormone content of the placenta by the reactions of the mammary glands¹ and uterus^{2, 3, 4} to injected extracts. It has been difficult to use such test reactions

quantitatively. In collaboration with Dr. Doisy and others the writer^{5, 6, 7} has shown that lipoid extracts of placenta will induce full oestrous growth in the vagina of the spayed rat and mouse, as will certain ovarian extracts. This reaction offers the following distinct advantages: (1) it affords several observations upon the living animal at definite intervals instead of a single one at autopsy for the determination of results; (2) it substitutes a maximum reaction which may be obtained in a shorter time interval (two days) for the partial reaction used in some of the earlier work; and (3) its clear cut end-point makes possible the quantitative utilization of this test for the biologic standardization of extracts in rat or mouse units.

Most of the extracts of placenta used in earlier work have been made from human material. Recently it was demonstrated that extracts of human placenta were as effective as follicular hormone from pig ovaries in inducing experimental menstruation in ovariectomized monkeys.⁸ Why the placenta should contain a substance similar to the essential hormone of the ovaries and to what extent these endocrine functions are related are fascinating problems. In the case of the opossum, from the nature of the embryonic membranes, such a hormone is probably not necessary to the mechanism of the pregnant cycle. The great diversity among mammals of structural modifications of the tissues making contact between mother and embryo would lead one to look for wide variation in this endocrine function. It is reasonable to suppose that some of the mammals might reproduce without it. Therefore the range of variation of hormone content of the placenta, or the chorionic membranes in animals where no definite placenta is formed, should be determined.

Working toward a solution of this problem, quantitative tests of the hormone content of various tissues of pregnancy from different species of mammals are being made as material becomes available. To date, extracts of placentas or chorionic membranes from the cow, sheep, horse, pig, dog, cat, rat and chick have been tested. Eventually enough data may accumulate to at least outline the range of hormone content of these tissues.

The human placenta at term, weighing from 460 to 700 or more grams may contain as much as 200 to 400 or even more rat units of active substance.⁶ A few analyses of placentas earlier in gestation have been made, and others are being added as specimens are received.⁹ Extracts of two human chorionic vesicles obtained from ectopic pregnancies of less than two months' standing, have given

positive tests in mice. These tests are interesting as the material extracted represents that derived entirely from the fertilized ovum. Positive tests have resulted also from extracts of material curetted from the uterus which showed placental structures on histologic examination.

The cotyledonary placentas of the cow* have shown the presence of this hormone in considerable quantity. The full term 'after-birth', representing of course only the embryonic part, has returned positive tests. Earlier in the gestation period it is possible to separate the maternal and embryonic parts of the cotyledons. This separation has been made on the killing floor of the packing house as soon as possible after the animals had been killed. While the embryonic part may be completely freed from maternal tissue, it is probable that tips of the chorionic villi may have been retained in the maternal pits. Both parts have returned analyses of nearly equal amounts of active substance.

Similar tests are at present being made of cotyledonary placentas from sheep.

Tests have also been made of full term chorions from the horse. This type of chorion with its diffuse arrangement of villi (representing of course only embryonic tissue) contains considerable amounts of active material.

Chorions of pigs taken during the latter half of the gestation period have so far returned uniformly negative tests. Extracts of the mucosa stripped from the uteri have been negative. It is worthy of note that in the pig the maternal embryonic relations are less intimate than those of the other mammals so far tested.

Analyses have been made of the zonular placentas of dogs and cats during the latter half of gestation. So far all tests have been negative. In some of these experiments the 'green borders' have been clipped off from the placentas before extraction because of the high solubility of their contents in lipid solvents. Additional tests are desirable before a definite statement of the absence of hormone from zonular placentas is made.

In a single experiment an extract of rat placentas from five litters removed late in gestation failed to give positive tests in mice.

In a preliminary series of tests of small amounts of tissue from chicks¹⁰ at different stages of incubation results have been negative.

It is highly desirable that additional tests of placentas and chor-

* I am indebted to Professor Andrew Uren of the Department of Veterinary Science, University of Missouri, for full term material from cows, horses, and sheep.

ionic membranes from various animals at different stages of gestation be made to complete a survey of the distribution and range of this material. Such studies should provide sound evidence for interpretation of the relation between the endocrine functions of ovaries and placenta in the higher mammals. The publication of quantitative data will be deferred until the completion of additional tests.

This is a preliminary report.

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- ¹ Lane-Clayton, J. E., and Starling, E. H., *Proc. Roy. Soc.*, 1906, lxxvii, 505.
² Aschner, B., *Arch. f. Gynaek.*, 1913, ic, 534.
³ Fellner, O. O., *Arch. f. Gynaek.*, 1913, c, 641.
⁴ Hermann, E., *Monat. f. Geburts. und Gynaek.*, 1915, xli, 1.
⁵ Allen, E., and Doisy, E. A., *J. Am. Med. Assn.*, 1923, lxxxii, 819.
⁶ Doisy, E. A., Ralls, J. O., Allen, E., Johnston, C. G., *J. Biol. Chem.*, 1924, lix, 711.
⁷ Allen, E., Doisy, E. A., and others, *Am. J. Anat.*, 1924, xxxiv, 133.
⁸ Allen, E., in press.
⁹ Allen, E., Pratt, J. P., and Doisy, E. A., *J. Am. Med. Assn.*, 1925, lxxxv, 399.
¹⁰ Allen, E., Whitsell, J. W., Hardy, J. W., and Kneibert, F. L., *Proc. Soc. Exp. Biol. and Med.*, 1924, xxi, 500.

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Degeneration in Vitro of Leucocytes and Connective Tissue Cells Under the Influence of Light.*

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In an attempt to keep tissue cultures of certain blood cells under constant observation, work was completely blocked by the exceedingly rapid and extreme degeneration of the cells. In this rapid degeneration all of the various types of leucocytes showed degenerative changes rather similar in character, which differed, however, in certain particulars, for each individual type of cell. Of the changes shown by the various types of leucocytes, those shown by the polymorphonuclear neutrophil were the most striking. The neutrophils rapidly became very amoeboid, and at the same time their cytoplasm became more fluid, as was shown by the exaggerated brownian movement of the cytoplasmic granules. Soon cellular movement ceased; the cells rounded and became spherical. The

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