

Bloor.² Total and free cholesterol were determined by the method of Okey³ as modified by Yasuda⁴ by precipitation and oxidation of the cholesterol as the digitonide. All experimental values are the averages of duplicate determinations. From the values obtained by the above procedures the further distribution of the liver lipids was found by calculation.

The fatty degeneration of the liver caused by chloroform is characterized by the microscopic appearance of globules of fat in the tissue. However, the results of analysis of the livers of white rats following severe chloroform injury, Table I(a), show that the total lipid content remains essentially normal, indicating that the appearance of globules of fat in the liver is due to a rendering visible of fat already present rather than an infiltration of fat from outside depots. The ratio of phospholipid to neutral fat was also found to be within the limits of that for normal liver tissue. Administration of large doses of insulin or fungus infection (*Aspergillus* and *Sporothrix*) likewise caused no significant changes in the amount and distribution of the liver lipids, Table I (b) and (c).

These results are at variance with those reported by Theis in that (1) the proportion of total lipid present as phospholipid in normal liver tissue is considerably higher than he found and (2) no displacement of the normal phospholipid: neutral fat balance takes place in the case of the white rat as the result of chloroform poisoning, insulin administration or fungus infection.

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Reactions of Immature Rabbit Ovary to Gonadotropic Extracts.

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The reactions of the prepubertal rabbit to gonad-stimulating extracts of anterior pituitary and of pregnancy urine have been extensively studied¹⁻³ but apparently no ovarian responses have been

² Bloor, W. R., *J. Biol. Chem.*, 1928, **77**, 53.

³ Okey, R., *J. Biol. Chem.*, 1930, **88**, 367.

⁴ Yasuda, M., *J. Biol. Chem.*, 1931, **92**, 303.

¹ Siegmund, H., *Arch. f. Gynäk.*, 1930, **142**, 702.

² Brindeau, A., Hinglais, H., and Hinglais, M., *Compt. rend. Soc. de Biol.*, 1932, **111**, 604.

observed in this species at periods prior to the appearance of antra-bearing follicles. Since the rat¹⁰⁻¹³ and guinea pig^{14, 15} show luteinization of the theca cells in response to injections of similar extracts at an age when the follicle granulosa is still non-reactive, it was of interest to reexamine the immature rabbit with particular reference to possible reactions of an analogous nature.

White rabbits from a single stock, and representing age groups of approximately 15, 30, 45, 60 and 90 days, were used. (Table I.) Each group comprised 2 litters totalling 10 to 14 females. At the beginning of the experiments, one ovary was removed from 5 or 6 of the animals of each group to provide control material. One-half of the animals received an extract of sheep anterior pituitary, and the remaining half an oestrin-free extract of pregnancy urine. One-quarter cc. of the pituitary extract was equivalent to one luteinizing rat unit; ascending doses from 0.5 to 1.5 cc. were injected subcutaneously twice daily for 14 days. Ten γ of the pregnancy urine extract (A.P.L.) represented one luteinizing rat unit; 100 such units were given intravenously once daily for 14 days.

A few animals were killed at intervals during the injections, but the majority were killed on the 15th day. The ovaries were sectioned serially, except in a number of instances where they were stained for lipoids by frozen section technique. In addition to these, a large number of control ovaries from mature rabbits (normal, treated and hypophysectomized) was studied.

The Normal Prepubertal Ovary. Two of the animals in the 45-day age group showed the beginnings of antra formation in a few follicles, but true vesicular follicles were first observed in 4 out of

³ Clauberg, C., *Z. f. Gynäk.*, 1932, **16**, 964.

⁴ Hertz, R., Hellbaum, A., and Hisaw, F. L., *PROC. SOC. EXP. BIOL. AND MED.*, 1932, **30**, 41.

⁵ Wolfe, J. M., and Ellison, E. T., *PROC. SOC. EXP. BIOL. AND MED.*, 1932, **29**, 600.

⁶ Hertz, R., and Hisaw, F. L., *Am. J. Physiol.*, 1934, **108**, 1.

⁷ Matsuura, Y., *J. Orient. Med.*, 1934, **20**, 77.

⁸ Aron, M., *Compt. rend. Soc. de Biol.*, 1931, **108**, 1216.

⁹ Watran, M., and Brabant, H., *Compt. rend. Soc. de Biol.*, 1931, **107**, 1418.

¹⁰ Noguchi, *Jap. J. Med. Sci. (Pharm. Sect.)*, 1931, **5**, 104.

¹¹ Selye, H., and Collip, J. B., *PROC. SOC. EXP. BIOL. AND MED.*, 1933, **30**, 647.

¹² Selye, H., Collip, J. B., and Thomson, D. L., *PROC. SOC. EXP. BIOL. AND MED.*, 1933, **30**, 780.

¹³ Selye, H., Collip, J. B., and Thomson, D. L., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 800.

¹⁴ Loeb, L., *Endocrinol.*, 1932, **16**, 129.

¹⁵ King, A. G., *PROC. SOC. EXP. BIOL. AND MED.*, 1933, **30**, 1182.

TABLE I.

Ages of Litters in days	Body Wt., Gm.	Wt. of Both Ovaries, Mg.
16, 17	158- 252	16- 28
32, 33	375- 492	22- 62
44, 47	535- 790	28- 38
62, 65	741-1425	32- 74
90, 92	1200-1650	56-176

5 of the 60-day-old animals. Also noted at this age, deep in the juxta-medullary region of the stroma, were a few of the large polyhedral interstitial cells which, in massive formations, make up the bulk and yellow color of the mature rabbit ovary. At 90 days the interstitial stroma formed the major part of the ovary. Though most of the cells of this tissue were still elongated and compactly arranged, a considerable number (including the still scattered polyhedral cells) took lipoid stains at this period. Also taking lipoid stains from this time forward were occasional cells of the theca interna of the larger follicles. Neither in the prepubertal nor adult rabbit, however, was follicular atresia in our material found to be associated, except occasionally, with the formation of "theca nests" such as those described by Wilkerson,¹⁶ and regularly seen in the rat and guinea pig.

Ovary of the Treated Prepubertal Rabbit. The youngest rabbits to respond to A.P.L. were the 45-day-old animals. Two of 7 treated animals showed several well-formed corpora lutea in each ovary. There were no signs of theca stimulation or of interstitial cell changes in these animals. The remaining 5 animals of this age showed neither granulosa nor thecal luteinization, nor were vesicular follicles present; clusters of large luteinized polyhedral cells, however, were present in the ovarian stroma. In both groups the uterus was still infantile at the end of the experiment.

In the 60-day-old A.P.L.-treated rabbits, 5 out of 6 animals showed well formed corpora lutea, together with numerous corpora hemorrhagica and cysts; these animals showed signs of recent progestational changes in the uterine mucosa. The remaining animal exhibited only marked hemorrhagic cyst formation, with very defective granulosa luteinization and a modified suboestric type of progestational uterine reaction. This type of reaction has been described by Wolfe and Ellison⁵ as due to the intravenous method of administering pregnancy urine, and probably represents an overstimulation of the follicle. Only one of the 6 animals of this group

¹⁶ Wilkerson, W. N., *Bull. Johns Hopkins Hosp.*, 1926, **38**, 339.

exhibited stromal changes. It is probably significant that in both 45- and 60-day-old rabbits, large interstitial cells appeared in numbers only when luteinization of the granulosa was defective.

At 90 days of age, the results of A.P.L. treatment approached those to be seen in similar treatment of mature females. Thus, 4 out of 5 animals showed extensive interstitial cell changes, though the granulosa luteinization varied widely in extent and character. Well formed corpora were found in 2 animals, and these showed typical progestational uteri. In the remaining 3, cystic and hemorrhagic corpora were observed, associated with modified progestational uterine reactions.¹⁷

First definite evidences of any effects from treatment with anterior pituitary extract were shown by 2 out of 5 of the 60-day-old rabbits. In these animals blood cyst formation and marked follicular atresia were apparent at the end of 15 days. Among 90-day-old animals similarly treated, one showed good corpora with typical uterine changes, while the remaining 4 showed extensive cyst and corpus hemorrhagicum formation with defective granulosa luteinization and greatly modified progestational uterine reactions. All 5 animals exhibited formation of large masses of luteinized polyhedral cells in the stroma.

The initial dose of pituitary extract given to the 60-day-old animals was probably subthreshold for this age, though potent in mature animals. Continued treatment with higher doses for as long as 14 days led to no eventual reactions either in follicle granulosa, theca or interstitial stroma, as is sometimes the case under similar circumstances in the rat and guinea pig. This was doubtless because of the rapid development of a refractory state.¹⁸ The sera of these animals inhibited gonadotropic reactions when given, along with pituitary extract, to immature rats in the manner previously described.¹⁹

Summary. The interstitial stroma of immature and juvenile rabbits responds to the injection of gonadotropic extracts by the formation of large polyhedral cells which take lipoid stains. In healthy and mature females, these cells are constantly present in great numbers and constitute the bulk of the mature ovary. Since they have been found (unpublished experiments) to disappear fol-

¹⁷ Leonard, S. L., and Hisaw, F. L., *Am. J. Physiol.*, 1930, **92**, 574.

¹⁸ Hisaw, F. L., Hertz, R., Hellbaum, A., and Fevold, H. L., *Anat. Rec.*, 1933, **55** S, No. 40.

¹⁹ Bachman, C., Collip, J. B., and Selye, H., *Proc. Soc. Exp. Biol. and Med.*, 1934, **32**, 544.

lowing hypophysectomy of the rabbit, they are apparently normally dependent to a degree upon the anterior pituitary. In the young animal they may form in response to gonad-stimulating substances before the follicle granulosa is capable of responding, and alternately, may fail to appear when the granulosa shows prompt and widespread luteinization during treatment. The possible effects upon them of continued gonadotropic treatment, such as can be seen in the theca of the rat, may be obscured by the rapid development of refractory states in the rabbit. Other features of their reactions indicate that they are not strictly comparable with the luteinized theca cells of the rat and guinea pig. In very young rabbits gonadotropic extracts may produce corpora lutea without eliciting a progestational uterine reaction.

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Blood Pressure Responses to Insulin.

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Kugelmann¹ reported 7 cases of angina pectoris who developed an elevation in blood pressure and substernal pain during hypoglycemia induced by the intravenous administration of insulin. The attacks of substernal pain and symptoms brought about by this procedure were similar to those which occurred spontaneously in these patients under ordinary circumstances. The studies of Kugelmann suggested to us using insulin hypoglycemia as a method of determining blood pressure responses. We have studied the blood pressure and blood sugar changes in normal individuals, in patients with essential hypertension, and in cases of Addison's disease following the intravenous administration of insulin. In each case a blood sugar determination (Folin Micro-method using venous blood) was made following a 12-hour fast; 15 units of insulin (Mulford) were then given intravenously, and subsequent blood sugar determinations made every 10 minutes for 90 minutes. To circumvent the effects due to emotional disturbances from repeated vena punctures only one puncture was made and the lumen of the needle kept

¹ Kugelmann, B., *Klin. Wochenschr.*, 1933, **12**, 1488.