Asplin and Boyland,[1] the chronic administration of sulfamethazine to young male rats caused atrophy of the testes and accessory glands. The basis for the discrepancy is not clear and it is not believed that the higher concentration of sulfamethazine in the diet (0.2% instead of 0.1%) of the experiments here reported accounts for the difference in results. Since atrophic changes of the testes were observed both grossly and microscopically in the present experiments in both the normal and hypophysectomized rats chronically fed with sulfamethazine, it would appear that the effect of the drug on the gonads of the young rats is one of direct inhibition. Although the interstitial tissue of the testes of the sulfamethazine-treated normal rats did not exhibit any histopathological changes, the atrophic changes in the accessory glands in these rats may still be considered to be secondary to a decreased secretory activity, without any detectable histological changes in the interstitial tissue.

Except that there was no significant change in weight (per 100 g body weight), the hyperplasia of the thyroid gland of the sulfamethazine-treated normal rats conformed with the observations of MacKenzies[4] and Astwood[5] who have reported that sulfonamides cause hyperplasia of thyroid glands in rats provided that the pituitary is intact. It remains to be determined, however, what significance should be attached to the apparent decrease in weights of the thyroid and adrenal glands in the hypophysectomized rats treated with sulfamethazine.

Summary. Chronic administration of sulfamethazine to normal male rats causes gross and microscopic atrophic changes in the testes, seminal vesicles and anterior prostate but a hyperplasia of the thyroid gland. The reported hypertrophy of the accessory organs[1] of the young male rats treated with sulfamethazine was not confirmed. When hypophysectomized rats were fed with sulfamethazine, only increased atrophy of the testes was manifested. The atrophic changes that occurred in the testes and accessory organs in normal rats, as well as the similar changes in the testes of the hypophysectomized male rats after chronic administration of sulfamethazine, are attributed to a direct effect of the drug on the testes.

The author wishes to express his indebtedness to Dr. H. B. van Dyke for suggestions and advice in carrying out the experiments, and Dr. P. E. Smith in examining the histopathological sections.

[4] MacKenzie, C. G., and MacKenzie, J. B., *Endocrinology*, 1943, **32**, 185.

[5] Astwood, E. B., Sullivan, J., Bissell, A., and Tyslowitz, R., *Endocrinology*, 1943, **32**, 210.

16529

Histochemical Variations in the Metrial Gland of the Rat During Pregnancy and Lactation.

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The metrial gland of the rat develops during pregnancy in association with the uterine blood vessels in the region of attachment of the mesometrium to the uterus. Some phases of the histology of the metrial gland have been investigated.[1-4] Since little is known concerning the possible endocrine function of the

* The author wishes to express his appreciation to Miss Barbara Piper for her technical assistance.

[1] Goldmann, E. E., *Beitr. z. klin. Chir.*, 1912, **78**, 1.

[2] Gérard, P., *C. R. Soc. de Biol.*, 1925, **93**, 457.

[3] Selye, H., and McKeown, T., *Proc. Roy. Soc.*, London, Series B, 1935, **119**, 1.

[4] Asplund, J., Borell, U., and Holmgren, H., *Z. f. mikr.-anat. Forsch.*, 1940, **48**, 478.

various parts of the rodent placenta, it was felt that a histochemical study of the metrial gland at various stages of pregnancy and lactation might give some indication of its function.

Methods. Virgin female rats were placed with males and their vaginal contents examined microscopically for spermatazoa not more than 18 hours later. Pregnancy was timed from the hour at which spermatazoa were found. Specimens were secured from over 61 rats from the fifth day of gestation to the twenty-second day of lactation. The metrial glands were fixed in Zenker-formol, picric-alcohol-formol, 10% neutral formalin, Carnoy's and Bouin's fluids. The following stains were employed: Masson, eosin and methylene blue and Best's carmine. Frozen sections of formalin-fixed tissues were studied for lipids by staining with Sudan red, Sudan black B, Nile blue sulfate and by the use of Schultz and plasmal reactions, polarizing microscopy and solubility tests. The Gomori[5] procedure was employed for alkaline phosphatase and Perls' reaction for iron.

Results. The history of the metrial gland during pregnancy and lactation may be divided into 3 overlapping periods, each of these being marked by one or more outstanding histochemical characteristics. These periods were indicated, respectively, by the presence of (1) basophilia, (2) eosinophilic granules and glycogen, and (3) lipids.

Period of basophilia. As early as the sixth day of pregnancy, many of the mesenchyme-like connective tissue cells of the metrial gland area became basophilic and began to round up. This basophilia increased significantly during the next several days and was accompanied by cellular enlargement and hyperplasia (Fig. 2). The nucleolus hypertrophied and came into contact with the nuclear membrane. Digestion with crystalline ribonuclease† showed the basophilia of both cytoplasm and nucleolus to be due to the presence of ribonucleic acid.

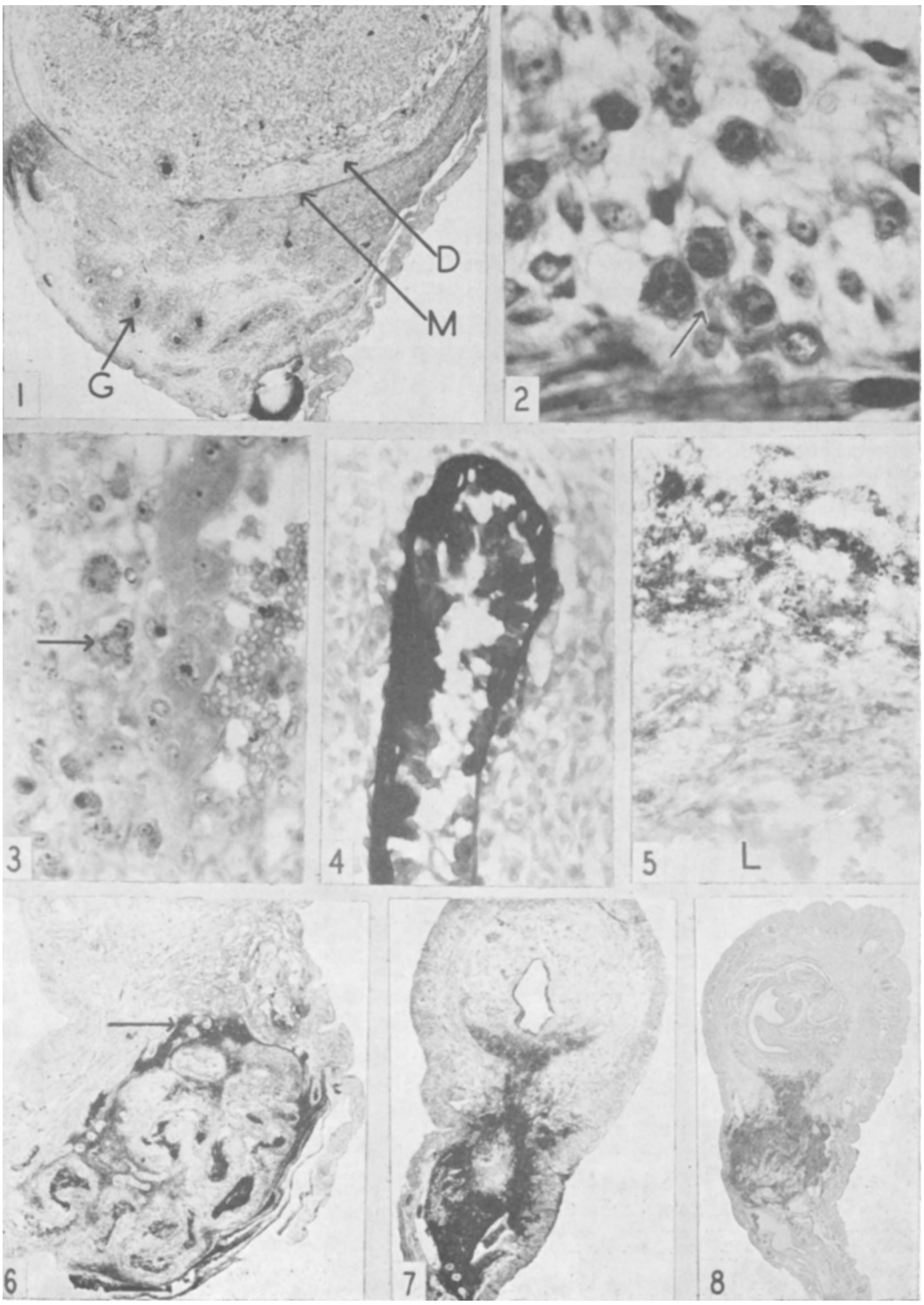
This period was comparatively brief, the basophilia declining coincidentally with the development of eosinophilic granules and glycogen in these basophilic cells. At the height of the second period, basophilic material remained only as scattered clumps at the periphery of the granules and no young basophilic cells in the pre-granulated stage were to be found.

It should be noted that the first evidence of formation of the metrial gland (basophilia) appeared almost contemporaneously with the development of the decidual reaction in the uterine mucosa. In fact, basophilic cells with characteristics like those described above for the metrial gland also were found scattered among the typical decidual cells of the decidua basalis. The decidual basalis and the metrial gland were anatomically close to each other, being separated by the discontinuous inner circular layer of the tunica muscularis (Fig. 1). Thus, it appears that the same stimulus causes the formation of both the metrial gland and the decidua, such a conclusion being borne out by the artificial induction of deciduoma and metrial gland by Selye and McKeown.[3]

Period of eosinophilic granules and glycogen. The first appearance of eosinophilic granules in the basophilic cells occurred in the decidua basalis on the eighth day. On the ninth and tenth days, such granulating cells were found also on the metrial gland side of the circular layer of the tunica muscularis but were still more numerous in the decidua basalis. Henceforth, the decidua basalis atrophied and lost its basophilia. Meanwhile, eosinophilic granules developed rapidly in the metrial gland, these cells becoming arranged in cuffs around the blood vessels of the mesometrial attachment (Fig. 1 and 3). Soon after the appearance of the granules, glycogen formed in the periphery of the cell with the eosinophilic granules being grouped close to the nucleus. Both constituents reached their peak at the thirteenth to fifteenth day. During this period, the endothelium of the metrial gland blood vessels became columnar in shape and faintly basophilic (Fig. 3), this transformation generally not extending completely

[5] Gomori, G., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 23.

† We wish to thank Dr. Moses Kunitz, the Rockefeller Institute for Medical Research, for supplying us with crystalline ribonuclease.


D
M
G
1
2
3
4
5
L
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7
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FIG. 1. Metrial gland (below M) at height of its development (fourteenth day of pregnancy). D—decidua basalis; M— inner layer of tunic muscularis; G—cuff of granulated cells around blood vessel.

FIG. 2. Basophilic cells (arrow). Eosin and methylene blue. Ninth day of pregnancy.

FIG. 3. Eosinophilic granules and glycogen vacuoles. Columnar endothelium. Thirteenth day of pregnancy.

FIG. 4. Alkaline phosphatase in columnar endothelium. Fourteenth day of pregnancy.

FIG. 5. Lipid at periphery of blood vessel. Nineteenth day of pregnancy. L—lumen.

FIG. 6. Lipid (arrow) at periphery of hyalinized vessel walls. Third day of lactation.

FIG. 7. Lipid (black) at fifth day of lactation. Sudan black B.

FIG. 8. Lipid at twenty-second day of lactation. Sudan red.

around the lumen. Intense alkaline phosphatase activity was demonstrated in such cells (Fig. 4). Subsequently, the eosinophilic granules and glycogen exhibited a steady decline and by the twenty-first day of pregnancy, both constituents were reduced significantly in amount.

The granulated cells met a varied fate. Some underwent necrosis *in situ*. Others were found in the lumina of the metrial gland blood vessels, apparently being carried toward the fetus.

Period of lipids. Lipid appeared on the seventeenth day of pregnancy at the periphery of the cuffs of granulated cells surrounding the metrial gland blood vessels (Fig. 5). Thus, it arose contemporaneously with the decline in glycogen and eosinophilic granules. The lipid increased slightly in quantity during the remainder of pregnancy and early lactation (Fig. 6) and remained located chiefly in the connective tissue between blood vessels. However, by the fifth day of lactation, the lipid-containing cells had formed a compact body (Fig. 7) containing an intricate network of capillaries. These cells were multiocular with vacuoles of varied size and possessed a central or slightly eccentric nucleus. Pigment appeared for which a hematogenous origin was indicated by a positive reaction for iron and failure to stain sudanophilically. In other cases, some of the pigment did appear to stain with Sudan black, indicating it to be lipochrome. The metrial gland remained without great change until the twenty-second day of lactation (Fig. 8).

The lipid gave a positive Schultz reaction, stained rose with Nile blue sulfate and recolorized the Schiff reagent without pretreatment with mercuric chloride. At least a part of it was birefringent and some of it showed the black cross of polarization. Much of it was acetone-soluble. This information suggests that the lipid was a mixture containing at least glycerides (some of which were unsaturated) and cholesterol or its esters.

Discussion. Ribonucleic acid was shown to be responsible for the early basophilia of the metrial gland. Cytoplasmic ribonucleic acid may be formed in the nucleolus[6] and be involved in protein synthesis.[7] Thus, the early prominence of basophilia in the metrial gland is to be correlated with rapid growth and hyperplasia of the organ and possibly also with the synthesis of whatever secretion may be represented by the eosinophilic granules of the second period.

Presumably the glycogen of the second period serves as nutriment for the embryo.[2,3] Alkaline phosphatase in the columnar endothelium probably assists in glycogenesis by splitting hexosephosphate.

The lipid may be of varied significance. First, some of its histochemical reactions resemble those which characterize the steroid-secreting endocrine glands. Possibly the metrial gland contributes to the steroid metabolism of the rat at the time of parturition and during lactation. Second, the lipid may have resulted from involution of the metrial gland. Third, in the structure of the lipid-containing cells, the positive reaction for cholesterol and its esters[8] and in its association with pigment, some of which may be lipochrome in type, the lipid of the metrial gland resembles brown fat. The physiology of

[6] Caspersson, T., and Schultz, J., *Proc. Nat. Acad. Science*, 1940, **20**, 507.

[7] Greenstein, J. P., *Advances in Protein Chemistry*, Academic Press, Inc., New York, 1944.

[8] Cramer, W., *Brit. J. Exp. Path.*, 1920, **1**, 184.

brown fat is poorly understood but an endocrine function has been suggested for it.[9]

Summary. During pregnancy and lactation the metrial gland is marked successively by the prominence of (1) ribonucleic acid, (2) eoinophilic granules and glycogen, and (3) lipids. These appear to be related, respectively, to growth and secretion, nutrition for the embryo and steroid metabolism.

[9] Long, J. A., *Anat. Rec.*, 1922, **23**, 107.

16530

Effect of Bacterial Endotoxins on Glycogen Synthesis.*

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It was found previously that the liver and muscle of rabbits which were poisoned with bacterial endotoxins showed a considerable depletion in glycogen.[1] Since the mechanism of this decrease in tissue glycogen was not known, further experiments were performed to investigate this problem. The experiments described in this paper deal with the effect of endotoxins on the synthetic process of glycogen formation from glucose and pyruvate *in vivo* and *in vitro*.

Experimental. The effect of Salmonella endotoxin on glycogen synthesis *in vivo* was studied in rats under the following experimental conditions:[2] Eighteen white rats, ranging in weight from 250 to 310 g, were starved for 48 hours. During this period the animals had free access to water. At the time of the experiment the animals were divided in 2 equal groups, one of which served as controls while the other was injected intraperitoneally with 2 ml (20 mg solid content) of Salmonella endotoxin per rat. It was found in preliminary experiments that this amount of endotoxin was lethal in 4-6 hours. One hour after injection of the endotoxin, 3 rats in each group were sacrificed and the liver glycogen determined.[3] At the same time, the remaining 12 rats were given 3 ml of saturated glucose solution by stomach tube. Two and 3 hours after the administration of glucose 3 animals in each group were sacrificed and their livers analyzed for glycogen. As indicated in Table I, the liver glycogen of the normal rats increased 6-8 fold in 2-3 hours, while the rats injected with endotoxin showed no appreciable increase. It is thought that this effect of the endotoxin is not a non-specific protein effect, since the injection of 20 mg bovine plasma protein per rat had no measurable effect on liver glycogen synthesis.

TABLE I.
Effect of Salmonella Endotoxin on Glycogen Synthesis in the Livers of Rats.

Time	Liver glycogen (mg/g) Normal	Poisoned*
Before glucose	1.74	0.18
	2.84	1.25
	2.21	0.97
2 hr after	16.2	0.39
	11.6	0.25
	15.8	0.36
3 " "	18.6	0.43
	16.9	0.44
	17.0	0.35

Each value obtained on one animal.
* Poisoned rats were injected with 2 ml (20 mg dry wt) of endotoxin one hour before glucose.

The effect of endotoxins on glycogen synthesis *in vitro* was studied in rabbit liver slices

* This investigation was undertaken and supported jointly by the U. S. Navy, Office of Naval Research, and the University of Chicago.

[1] Kun, E., and Miller, C. P., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 221.

[2] Barron, E. S. G., personal communication.

[3] Unbreit, W. W., Burris, R. H., and Stauffer, J. F., *Manometric Techniques and Related Methods for the Study of Tissue Metabolism* (Burgess Publishing Company), 1946, p. 170.