the degree of hypoglycemia even in the presence of a large liver glycogen store. Experiments to clarify this point are currently in progress.

Summary. Experiments in rabbits and mice have demonstrated that the presence of glucagon in insulin solutions in ratios of from 1:100 to 1:1 did not influence the degree of hypoglycemia produced by the insulin, and consequently did not alter the potency of the insulin preparation as determined by the mouse convulsion assay.

The authors wish to thank Mr. Zane Frank and Mr. Darrell Caldwell for the blood sugar determinations on rabbits, and Mr. R. Kennedy for the blood sugar determinations and insulin assays on mice.

1. Kimball, C. P., and Murlin, J. R., *J. Biol. Chem.*, 1923, v58, 337.

2. Sutherland, E. W., and Cori, C. F., *ibid.*, 1948, v172, 737.

3. Heard, R. D. H., Lozinski, E., Stewart, L., and Stewart, R. D., *ibid.*, 1948, v172, 857.

4. de Duve, C., Hers, H. G., and Bouckaert, J. P., *Arch. Internat. Pharmacodyn.*, 1946, v72, 45.

5. Olsen, N. S., and Klein, J. R., PROC. SOC. EXP. BIOL. AND MED., 1947, v66, 86.

6. de Duve, C., *Lancet*, 1953, v2, 99; Vuylsteke, C. A., and de Duve, C., *Arch. Internat. Pharmacodyn.*, 1953, v61, 275.

7. Hagedon, H. C., and Jensen, B. N., *Biochem. Z.*, 1923, v135, 46.

8. Emmens, C. W., *Hormone Assay*, Academic Press, N. Y., 1950, Chapter II.

9. Haslewood, G. A. D., and Strookman, T. A., *Biochem. J.*, 1939, v33, 920.

10. Staub, A., Sinn, L., and Behrens, O. K., *Science*, 1953, v117, 628.

Received February 25, 1954. P.S.E.B.M., 1954, v85.

Composition of Rat Seminal Vesicles and Effects of Testosterone Propionate on Lipid Distribution.* (20935)

L. F. CAVAZOS, J. C. PORTER,† AND R. M. MELAMPY.

From Iowa Agricultural Experiment Station, Iowa State College, Ames.

Moore, Hughes, and Gallagher(1) have pointed out the usefulness of rat seminal vesicles in investigations dealing with the physiological effects of male sex hormone. Furthermore, biochemical and histochemical studies have been carried out dealing with the action of this hormone on various aspects of the metabolic processes of the gland such as protein synthesis(2,3), respiratory metabolism(4), fructose production(4), and the activities of various enzymes(5). According to Roberts and Szego(6), there is little information available relative to the lipid metabolism of the male reproductive target organs. Mann(7) has described 3 types of cells in the epithelium of the seminal vesicles of the bull. The most numerous, type A, is columnar and borders on the lumen. The cells of B type are located basally and are characterized by the presence of lipid. The C type is the least numerous and occurs among the A cells. The B type described by Mann(7) is probably the lipid-containing cell mentioned by Limon(8). It is of interest that Limon(8) reported the absence of lipid in the seminal vesicle of a bull calf of 3 months of age. Akutsu(9) observed the presence of lipid material in the epithelium of rat seminal vesicles.

Data are presented dealing with changes in chemical composition, including dry matter, total N, and total lipid, of rat seminal vesicles during sexual development. In addition, observations are included on sudanophilia and phospholipid distribution in this organ in intact as well as castrates and hormone-treated castrates.

Materials and methods. Rats of the Holtzman-Rolfsmeyer strain were used and were fed Purina Laboratory chow supplemented

* Journal Paper No. J-2488 of the Iowa Agric. Exp. Station, Ames, Ia. Project 936.

† Present address: Department of Physiology, Southwestern Medical School of University of Texas, Dallas.

TABLE I. Changes in Weight, Total Nitrogen and Total Lipid of Rat Seminal Vesicles during Sexual Development.

Group No.	No. of animals	Body wt, g	Seminal vesicles				
			Wet wt, mg	Dry wt, mg	Dry wt, %	Total N., mg	Total lipid, mg
1	10	77* ± 1.3	9.6	2.1	21.9	.3	.1
2	10	94 ± 1.8	11.3	2.2	19.5	.3	.1
3	11	148 ± 2.7	48.0* ± 4.6	10.0* ± 1.0	20.8	1.3	.6
4	11	189 ± 1.4	271.0 ± 25.7	57.0 ± 6.0	21.0	8.6	1.7
5	12	256 ± 4.2	595.0 ± 25.4	141.0 ± 6.2	23.7	21.4	3.5
6	9	298 ± 3.5	693.0 ± 29.7	166.0 ± 8.8	24.0	24.9	3.8
7	10	356 ± 4.2	1401.0 ± 84.2	341.0 ± 21.4	24.3	51.8	5.5
8	8	386 ± 5.1	1602.0 ± 50.2	394.0 ± 41.0	24.9	59.5	4.7

* Mean and stand. error.

with raw carrots. A total of 81 rats divided into 8 groups was used to obtain data relative to the composition of the seminal vesicles during development. Animals were placed in various groups according to body weight as indicated in Table I. Dry matter of the seminal vesicles was determined by heating samples at 105°C overnight in an electric oven. Total nitrogen analyses were made by a standard micro-Kjeldahl procedure. Dried glands were extracted with ethyl alcohol followed by ethyl ether in a Soxhlet extractor and the lipid was determined gravimetrically following solvent removal on a steam bath. Lipid distribution in the seminal vesicles of rats was studied by histochemical procedures. A total of 27 animals was used in this phase of the investigation as follows: 3 intact sexually mature males; 12 castrates, 2, 5, 10, and 20 days after gonadectomy; and 12 castrates receiving subcutaneously 500 μg of testosterone propionate (Peranderen) daily for 2, 5, 10, and 20 days. The seminal vesicles of animals receiving hormone were allowed to atrophy for a 20-day period following orchidectomy prior to treatment. At time of castration, these animals weighed 250-300 g. Tissues were fixed in 10% neutral formalin and embedded in gelatin. Frozen sections were cut at 10 μ and stained with Sudan black B and oil red O. In addition, tissue was fixed in formaldehyde calcium and weak Bouin's fluid as recommended by Baker(10) for the acid hematin test for phospholipids.

Results. Data obtained relative to changes in weight, total N, and total lipid of the seminal vesicles of the rat during growth are presented in Table I.

The data presented in Table I indicate that there was no significant difference between the dry weights of the seminal vesicles of Group 1 and Group 2; however, the mean body weight of the latter was 17 g greater than the former. It is suggested that the increase in the weight of the seminal vesicles of Group 3 was due to the onset of secretory function of the testes. This agrees with the findings of Moore and Price(11) who reported that rats were approximately 35 days old before the testes produced a sufficient amount of hormone to induce a seminal vesicle response. Likewise, Callow and Deanesley(12) reported that the strain of rats used by them weighed 100 g before the seminal vesicle weight began to increase. While the mean body weight of Group 5 was approximately 3 times greater than Group 1, the seminal vesicle dry weight of Group 5 was 67 times greater. Also, the body weight and seminal vesicle dry weight of Group 8 increased 5-fold and 188-fold, respectively. The increase in percentage dry weight between Groups 5 and 8 as compared to that between Groups 1 and 4 was due to an accumulation of secretion inasmuch as these data were based upon dry-weight determinations of the glands and their contents. The total nitrogen content of these glands and their secretion increased 200-fold between Group 1 and Group 8; whereas, the total lipid increased approximately 50 times. The lipid in these glands was present only in the tissue as it has been demonstrated that the secretion is fat free(2).

The average lipid content of the seminal vesicles of 21 intact males weighing between 250 and 300 g was 3.7 mg per gland. On a

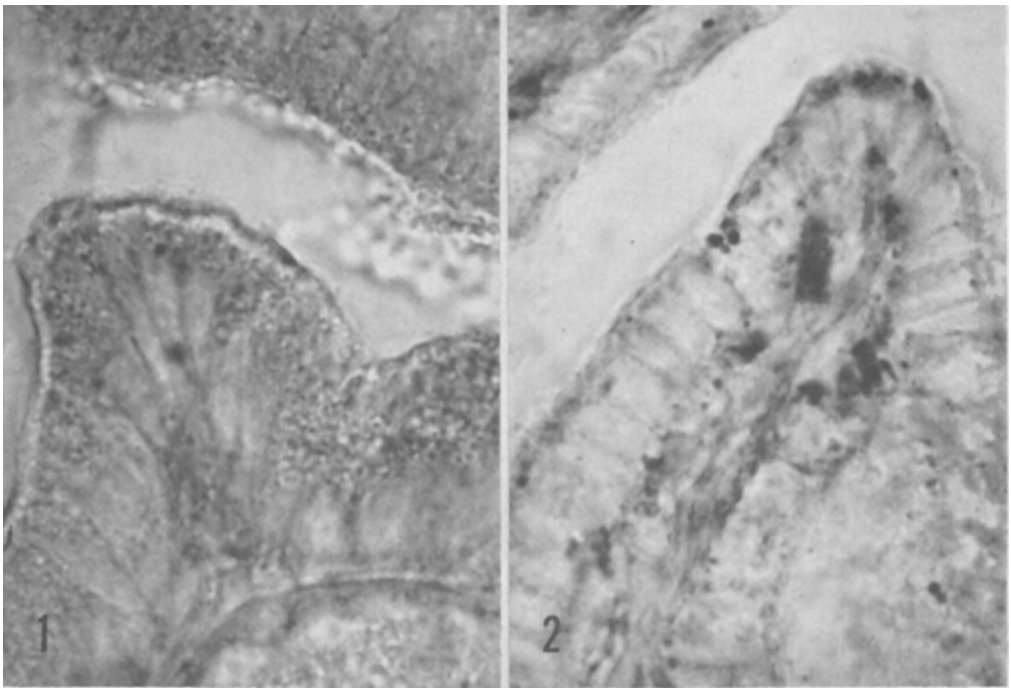

FIG. 1. Diffuse sudanophilia in cytoplasm of secretory epithelium of rat seminal vesicles of intact animal following Sudan black B. Note non-reacting secretory granules. (×600)
FIG. 2. Lipid distribution in epithelium and interacinar stroma of 20-day castrate rat. (×600)

dry-weight basis this was 2.4% total lipid. Moreover, a diffuse sudanophilia was observed in the cytoplasm of the secretory epithelium of rat seminal vesicles of similar intact animals following Sudan black B as shown in Fig. 1. Most of this coloration was present in the apical areas. Numerous non-reacting secretory granules, surrounded by clear unstained halos, were present. It is important to stress that these granules were comparable in appearance to those observed in sections extracted with ethyl ether-chloroform mixture. The nuclei were unstained as indicated in Fig. 1. Numerous small droplets of lipoidal material were noted in the lumen of arterioles and venules and in a few cases, lipid was present in the interacinar stroma. A strong reaction for phospholipids was demonstrated in the secretory granules of the epithelium whereas other components of this tissue were unreactive with Baker's acid hematin method. A positive test was found in most erythrocytes. No lipid was demonstrated in the secretion in the lumen of the gland (Fig. 1). This confirms results of earlier work dealing with the chemical composition of the secretion(2).

Two days following castration the secretory epithelium showed indications of transition from the columnar to the cuboidal type. As in the intact animals, the greatest amount of sudanophilia was demonstrated in the apical portion of the cytoplasm, but a weak reaction was noted in the basal region of these cells. This sudanophilic material appeared, in many instances, as more discrete droplets than that observed in the intact animals and only occasionally were there fine fat globules present in the interacinar stroma. Reactive, small secretory granules with Baker's method were present in the apical cytoplasm of the epithelium at this time. In addition, droplets of phospholipid were observed in the basal end of these cells. The secretory epithelium of 5-day castrates had changed to a cuboidal type. No secretory granules were visible in the cytoplasm, and only a weak sudanophilia was seen in the apical end of the cell. The involution of the secretory epithelium follow-

ing castration was accompanied by an apparent increase in sudanophilic lipid at the basal region. The marked accumulation of lipid observed may have resulted from either lipid synthesis and deposition in this region, or the dispersed sudanophilic material may have collected basally during cytoplasmic regression. A fine reacting granulation was noted in the connective tissue of the interacinar stroma. Cytological appearance after 10- and 20-days castration was similar to that of the 5-day animal. Fig. 2 shows the lipid distribution in the epithelium and interacinar stroma of a 20-day castrate. It is of interest that the average lipid content of the seminal vesicles of 3 castrate males weighing approximately 300 g was 2.9 mg per gland or 12.9% total lipid. These animals had been gonadectomized 20 days. In the 5-day castrate there appeared to be an increase in the amount of phospholipid at the basal portion of the secretory epithelium. However, in the 10- and 20-day gonadectomized animals, a small amount of reactive material was uniformly present in the basal portion of the cells and in a few cases, apparent in the apical region.

Two days following hormone treatment a diffuse sudanophilia was again observed in the apical cytoplasm of the secretory epithelium. During this interval these cells had increased in height and a few secretory granules were visible. In some cases secretory granules have not been observed in the seminal vesicles of the rat 2 days following hormone treatment (1,3). However, this may be the result of the different fixing and staining technics employed. Occasionally droplets of material staining with Sudan black B were localized at the basal end of the epithelium and a few, fine granules were dispersed in the interacinar stroma. A positive phospholipid reaction was observed in the few secretory granules which were visible in the cytoplasm at this time, and occasionally droplets of positive material were observed in the basal portion of these cells. A diffuse cytoplasmic sudanophilia was noted in the apical and basal regions of the secretory epithelium in the 5-, 10-, and 20-day hormone treated castrates. The intensity of this reaction was comparable to that observed in intact males. Furthermore, the increase in number and size of secretory granules in these injected animals was accompanied by an associated phospholipid reaction. These results suggest that phospholipids are in some way involved in the active metabolism of the secretory epithelium, particularly the granules. In all stages described, the nucleus was unreactive (Fig. 1 and 2). Results obtained with oil red O were similar to those reported for Sudan black B. Treatment of sections with 1:1 ethyl ether-chloroform mixture removed most of the reactive lipid in this tissue.

Summary. In sexually immature rats (wt 77 g) the seminal vesicles weighed 2.1 mg (dry wt) and contained 0.3 mg total N and 0.1 mg lipid, whereas in sexually mature animals (wt 386 g) the glands weighed 394 mg with 59.5 mg N and 4.7 mg lipid. A diffuse cytoplasmic sudanophilia was observed in the columnar epithelium of the seminal vesicles of sexually mature males. Following castration there was a marked accumulation of lipid in the basal end of the cuboidal cells. The administration of testosterone propionate caused the diffuse sudanophilia to appear. Phospholipid was observed in the secretory granules. A marked reduction in phospholipid reaction was observed following gonadectomy due to the disappearance of the granules. A marked increase in the granules and associated phospholipid reaction was noted following hormone replacement.

Acknowledgement is made of support by research grant, National Cancer Inst. of Nat. Inst. of Health Service, Department of Health, Education, and Welfare. The authors acknowledge the technical assistance of Barbara W. Starks.

1. Moore, C. R., Hughes, W., and Gallagher, T. F., A. J. Anat., 1930, v45, 109.

2. Porter, J. C., and Melampy, R. M., *Endo.*, 1952, v51, 412.

3. Melampy, R. M., and Cavazos, L. F., *ibid.*, 1953, v52, 173.

4. Rudolph, G. G., and Samuels, L. T., *ibid.*, 1949, v44, 190.

5. Davis, J. S., Meyer, R. K., and McShan, W. H., ibid., 1949, v44, 1.

6. Roberts, S., and Szego, C. M., *Physiol. Rev.*, 1953, v33, 593.

7. Mann, T., Davies, D. V., and Humphrey, G. F.,

J. Endo., 1949, v6, 75.
8. Limon, M., *J. de L'Anat. et de Physiol.*, 1901, v37, 424.
9. Akutsu, S., *Arch. Physiol.*, 1903, v96, 555.
10. Baker, J. R., *Q. J. M. Sci.*, 1946, v87, 441.
11. Moore, C. R., and Price, D., *Endo.*, 1937, v21, 313.
12. Callow, R. K., and Deanesley, R., *Biochem. J.*, 1935, v29, 1424.

Received March 1, 1954. P.S.E.B.M., 1954, v85.

A Practical Laboratory Preparation of Avidin Concentrates for Biological Investigation. (20936)

FREDERICK G. DHYSE. (Introduced by Roy Hertz.)
From the National Cancer Institute, Bethesda, Md.

The production of a biotin deficiency in laboratory animals or in patients requires the ingestion of large quantities of egg white. For example, one investigator(1) gave each patient 36-42 egg whites per day for 6 months. Inasmuch as the anti-biotin activity of egg white is due to avidin(2) which constitutes less than 0.05% of egg white proteins(3), a convenient method of concentrating this activity is desirable.

The objective of this work was a simple method of preparing substantial amounts of avidin concentrate, adequate in activity for animal experimentation and clinical trial, rather than a method for obtaining a small amount of "pure" avidin for chemical and physical analysis.

Method. Four hundred grams of dried egg white* was dissolved in 4 liters of distilled water and adjusted to *pH 5.1* with one normal hydrochloric acid. Then 4½ liters of acetone were added and after stirring for 15 minutes more, the batch was filtered on a 24 cm diameter Buchner funnel using Whatman No. 52 paper. The acetone filtrate was discarded and the filter cake suspended in 2 liters of distilled water, stirred 15 min. and filtered. The wash water filtrate was discarded and the precipitate was extracted in 4 liters of 1% sodium chloride for one hour (the batch was usually allowed to stand overnight at this point). The batch was filtered and the saline filtrate used to extract a second 400-g portion of egg white treated in the same manner as the first. The batch was filtered and a fresh 4-liter portion of saline was used to extract each cake in turn. The saline filtrates (representing 800 g of dried egg white), were poured into 15-inch lengths of dialysis tubing,† 3 inches in diameter. These "sausages" were then put into tall (6 x 18 in.) pyrex battery jars filled with distilled water. The dialysis was carried out in a refrigerator at about +5°C, and is continued for 2 days with twice daily changes of distilled water. The active fraction precipitates at this time. The contents of the dialysis bags were rinsed out into a 2-gallon pyrex battery jar. However, most of the precipitate was quite sticky and clung to the tubing, but it could be removed by rinsing the bags with 3 successive 100 ml portions of 1% NaCl in which the precipitate is completely soluble. The saline rinsings were kept separate from the water rinsings. About 50 g of filter aid‡ was then stirred into the water rinsings from the dialysis bags to collect the suspended avidin. The Celite was filtered out and suspended in the saline rinsings, stirred for about one-half hour and filtered. The salt solution which contains the avidin activity was mixed with an equal volume of cold ethyl alcohol which caused precipitation of the protein but not of the salt. The precipitate was removed by centrifugation in a refrigerated centrifuge and after washing once with 50% alcohol. The precipitate freeze-dried to a powder.

* Product of Wilson Co., Chicago, Ill.

† Cellulose Sausage Casings-Visking Corp., Chicago, Ill.

‡ Celite analytical filter aid—Johns-Manville Co. A Diatomaceous silica filter aid.