Effect of Hypophysectomy and Replacement Therapy on Fatty Acid Metabolism in the Rat Testis

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1. The influence of pituitary gonadotrophins and of testosterone on the conversion of linoleic acid into other polyunsaturated fatty acids by rat testicular tissue was studied. 2. In immature hypophysectomized rats, follicle-stimulating hormone caused a threefold increase in the incorporation of radioactivity from [1^{14}C]linoleic acid into testicular lipids; the distribution of ^{14}C in the polyunsaturated fatty acids, however, was not significantly affected. 3. In mature hypophysectomized rats, the hormonal treatments had less pronounced effects on ^{14}C incorporation into testicular lipids, but caused a significant increase in the percentage of ^{14}C incorporated into polyunsaturated fatty acids of the $\omega-6$ series, luteinizing hormone and testosterone having the more pronounced influences. 4. A time-course study of the appearance of radioactivity in the ejaculated spermatozoa of rabbits, after they had been given a tracer dose of [1^{14}C]linoleic acid, indicated that incorporation of radioactivity into spermatozoa occurred during all stages of spermatogenesis.

The importance of the polyunsaturated fatty acids in testicular development and spermatogenesis is well recognized (Kirschman & Coniglio, 1961; Ahluwalia & Holman, 1966; Davis, Bridges & Coniglio, 1966), but the significance and the exact nature of their involvement are still uncertain. The characteristic increase in the concentration of docosapentaenoic acid in the maturing rat testis (Davis *et al.* 1966) suggests some hormonal control, direct or indirect, but little work has been done in this field. Finerty, Klein & Panos (1957) reported that testosterone corrected the degenerative changes in germinal epithelium induced by fat deficiency. However, in view of the earlier report by Panos & Finerty (1954) that the Leydig cells and accessory sex organs remain intact in fat-deficient rats, despite the degeneration of germinal epithelium, the androgenic influence is still uncertain. Some observations on the influence of hormones on the biosynthesis of some testicular lipid constituents have been made (Gambal & Ackerman, 1967), but polyenoic fatty acids, which seem to be more fundamentally involved in sperm maturation and development (Kirschman & Coniglio, 1961; Ahluwalia & Holman, 1966), were not studied. The present paper reports some observations on the polyenoic fatty acid content of rat testis under the influence of testosterone and the pituitary gonadotrophins.

MATERIALS AND METHODS

Male Sprague-Dawley rats, hypophysectomized at 4 weeks of age, were obtained from the Hormone Assay

Laboratory, Chicago, Ill., U.S.A. Mature male rats of the same strain, each weighing 200–225 g., were hypophysectomized by the intra-aural technique. Five rats were killed immediately after receipt of the shipment or immediately after hypophysectomy, and their testes and seminal vesicles were removed and weighed. The rest of the animals were caged at 22° and were fed *ad libitum* on commercial rat chow.

After the rats had been housed for 2 weeks under laboratory conditions, another five animals were killed and their testes and seminal vesicles removed and weighed. The rest of the animals were then divided into five groups of six animals each receiving the following injections: (1) corn oil (Mazola corn oil from Corn Products Co., New York, N.Y., U.S.A.); (2) FSH* (obtained from the Endocrinology Study Section, National Institutes of Health, Bethesda, Md., U.S.A.); (3) LH (PLH obtained from Armour-Baldwin Laboratories, Omaha, Neb., U.S.A.); (4) FSH and LH; (5) TP (obtained from K & K Laboratories Inc., Plainview, N.Y., U.S.A.). The hormones were suspended or dissolved in corn oil, each injection of 0.5 ml. containing 2 mg. of FSH, 40 i.u. of LH or 250 μg . of TP as appropriate. The hormones were injected subcutaneously at 0, 24 and 48 hr. Each animal was given 10 μC (0.2 ml.) of methyl [1^{14}C]linoleate (radiopurity 98% after purification by preparative thin-layer chromatography in AgNO_3 -impregnated silica gel) at 66 hr., and the animals were decapitated at 72 hr. The testes and seminal vesicles were quickly removed, weighed and stored at 20° until analysed. Remnants of pituitary were looked for in the decapitated animals and the few animals in which the slightest traces could be detected were rejected.

The extraction of the total lipids, separation into lipid

* Abbreviations: FSH, follicle-stimulating hormone; LH, luteinizing hormone; TP, testosterone propionate.

classes and the analyses of fatty acids were done by methods described by Goswami & Coniglio (1966). The prostaglandins from the seminal vesicles were extracted by the method of Green & Samuelsson (1964) and samples of the extracts were used for scintillation counting. The prostaglandins were not fractionated further because of the small amounts available.

To follow the appearance of radioactivity from [^{14}C]-linoleic acid in ejaculated sperm, two white New Zealand rabbits, weighing about 3 kg. each, were given $100\mu\text{C}$ of [^{14}C]linoleic acid by stomach tube, and semen collection was started on the next day. Two successive ejaculates, 10–20 min. apart, were collected by artificial vagina from each rabbit every second day. Immediately after ejaculation, the gel plug was removed and the semen centrifuged at 3000 rev./min. (1300g) for 10 min. in a Clay-Adams clinical centrifuge. The sperm were washed twice by resuspending them in Krebs-Ringer 0.1M-phosphate buffer, pH 7.4 (Cohen, 1957), and recentrifuging. The washings were added to the seminal plasma. The lipids were extracted from both the sperm fraction and the seminal plasma fraction by the Folch procedure (Folch, Lees & Sloane-Stanley, 1957). The extracts were evaporated to dryness, the residue was redissolved in light petroleum (b.p. 40–60°) and samples of the extracts were transferred to scintillation vials for counting.

RESULTS

With immature rats, there was a 4% increase in the mean body weight of the animals during the 2-week adaptation period between hypophysectomy and replacement therapy (Table 1). The hormonal treatments did not have any effect on this growth rate. There were no significant alterations in the testicular weights of the hypophysectomized immature rats during the adaptation period. Though there were great individual variations, the testicular weights were in general the highest in the FSH-treated and the FSH-LH-treated groups. All the hormonal treatments had significant effects on the seminal vesicle weights.

In the mature rats, the body weights decreased

by about 20% during the 2-week period after hypophysectomy. Compared with the control group, there were increases in testicular weight after LH treatment (30% increase) and FSH-LH-treatment (35% increase), but not after FSH treatment alone; TP treatment decreased testicular weight by about 33%. FSH treatment produced a significant increase (39%) in seminal vesicle weight, but LH treatment led to a much greater increase (125%). The effect of FSH-LH treatment was slightly but not significantly less than that found after LH alone. TP treatment led to a 220% increase in the seminal vesicle weight.

Effect of hormones on the incorporation of ^{14}C from [^{14}C]linoleic acid into testicular and seminal-vesicle lipids. Table 2 shows the incorporation of ^{14}C from [^{14}C]linoleic acid into testicular lipids and seminal-vesicle prostaglandins. LH treatment gave an increase of 77%, and FSH treatment gave an increase of 198%; FSH-LH treatment gave variable results between 75% and 125%. The increased incorporation with TP (29%) was not significant. Variations between animals within a group were high in the LH-treated and TP-treated animals, but the effect of FSH was more uniform. In mature hypophysectomized rats, the increases after hormone treatment were not significant. Marked FSH stimulation of ^{14}C incorporation into prostaglandins occurred in both immature and mature rats. The amount of radioactivity in prostaglandins after treatment with TP or LH was lower than that obtained by FSH treatment. This suggested that stimulation of seminal vesicular growth by testosterone is not necessarily associated with increased prostaglandin synthesis.

Of the three lipid components in the testicular tissue that were examined, the major incorporation (74–86% of the total) was into the phospholipids, and the various hormonal treatments did not significantly affect the ^{14}C distribution. Only the

Table 1. *Effects of hormonal treatments on body weight and on testis and seminal vesicle weights of hypophysectomized rats*

Mean values $\pm$ S.E.M. are given for each group, and the results are expressed as percentages of the values found for the first group.

Group	Immature weanling rats			Mature rats		
	Body wt.	Testis wt.	Sem. ves. wt.	Body wt.	Testis wt.	Sem. ves. wt.
Immediately after hypophysectomy	100 $\pm$ 3.8	100 $\pm$ 4.6	100 $\pm$ 2.9	100 $\pm$ 3.1	100 $\pm$ 3.7	100 $\pm$ 4.2
2 weeks after hypophysectomy	104 $\pm$ 3.9	93 $\pm$ 6.8	91 $\pm$ 7.3	81 $\pm$ 7.3	55 $\pm$ 9.9	42 $\pm$ 5.6
Control	108.6 $\pm$ 9.1	96 $\pm$ 8.1	93 $\pm$ 7.5	83 $\pm$ 6.9	58 $\pm$ 8.9	44 $\pm$ 8.9
FSH	104.3 $\pm$ 7.6	125.4 $\pm$ 11.6	198 $\pm$ 12.9	85 $\pm$ 8.1	66.5 $\pm$ 11.6	61 $\pm$ 8.0
LH	104.1 $\pm$ 6.9	100.8 $\pm$ 5.1	268 $\pm$ 14.1	87 $\pm$ 7.1	71.6 $\pm$ 8.6	99 $\pm$ 7.9
FSH-LH	106.5 $\pm$ 7.1	111 $\pm$ 10.6	196 $\pm$ 11.2	87 $\pm$ 6.9	83 $\pm$ 7.0	83 $\pm$ 9.1
TP	109 $\pm$ 7.3	87.1 $\pm$ 12.1	271 $\pm$ 14.6	89 $\pm$ 9.1	36.8 $\pm$ 6.9	141 $\pm$ 12.8

Table 2. *Effects of hormonal treatments on the incorporation of radioactivity from [1-¹⁴C]linoleic acid into testicular lipids and seminal-vesicular prostaglandins of hypophysectomized rats*

The results are expressed as counts/min./mg. of tissue. Each result is the mean $\pm$ S.E.M. of values for a group of six animals. n.s., Not significant.

Group	Testicular lipids		Seminal-vesicular prostaglandins	
	Immature	Mature	Immature	Mature
Control	2.76 $\pm$ 0.81	1.94 $\pm$ 0.09	Trace	Trace
FSH	8.22 $\pm$ 0.91	3.01 $\pm$ 0.41	1.01 $\pm$ 0.03	5.33 $\pm$ 1.10
LH	4.88 $\pm$ 1.68	2.58 $\pm$ 0.63	0.81 $\pm$ 0.01	2.01 $\pm$ 0.86
FSH-LH	5.11 $\pm$ 1.38	2.15 $\pm$ 0.38	0.89 $\pm$ 0.03	2.67 $\pm$ 0.82
TP	3.55 $\pm$ 1.01	1.05 $\pm$ 0.08	0.85 $\pm$ 0.05	2.00 $\pm$ 0.41
<i>P values</i>				
Control versus FSH	< 0.001	n.s.	FSH versus LH	< 0.01
Control versus LH	< 0.05	n.s.	FSH versus TP	< 0.01
Control versus TP	n.s.	n.s.		

Table 3. *Effects of hormonal treatments on the incorporation of radioactivity from [1-¹⁴C]linoleic acid into various fatty acids in the phospholipid fraction of testes from hypophysectomized immature rats*

The results are expressed as percentages of the total radioactivity in the phospholipid fatty acids. Each result is the mean $\pm$ S.E.M. of values for a group of six animals. The first figure of the fatty acid designation represents the number of carbon atoms and the second figure the number of double bonds. The positions of the double bonds in the 20:3 acid are given in parentheses. The concentrations of, and the radioactivity incorporation into, the fatty acids of the FSH-LH-treated group were not significantly different from the values in the FSH-treated group and they are therefore not shown.

Fatty acid	Group ...	Radioactivity in fatty acid (% of total)			
		Control	FSH	LH	TP
Total < 18:2		19.3 $\pm$ 2.1	25.4 $\pm$ 2.1	20.8 $\pm$ 3.2	21.7 $\pm$ 2.3
18:2		51.8 $\pm$ 5.6	46.8 $\pm$ 5.0	43.6 $\pm$ 5.7	41.4 $\pm$ 4.5
20:2		5.7 $\pm$ 1.2	4.8 $\pm$ 1.1	9.0 $\pm$ 1.4	8.2 $\pm$ 1.9
20:3 (8,11,14)		2.0 $\pm$ 1.0	3.2 $\pm$ 1.0	5.8 $\pm$ 1.4	6.3 $\pm$ 1.3
20:4		7.1 $\pm$ 2.0	8.9 $\pm$ 1.7	10.2 $\pm$ 2.8	10.1 $\pm$ 1.9
22:4		4.4 $\pm$ 1.0	3.6 $\pm$ 1.0	3.5 $\pm$ 1.1	3.0 $\pm$ 0.9
22:5		0.5 $\pm$ 0.1	0.8 $\pm$ 0.0	1.1 $\pm$ 0.2	1.5 $\pm$ 0.3
24:5		—	2.4 $\pm$ 0.7	5.3 $\pm$ 1.1	4.9 $\pm$ 1.0
Total > 18:2		22.0	27.2	35.3	34.0
<i>P values for total < 18:2</i>					
		FSH versus			
		Control	LH versus FSH	TP versus FSH	
		< 0.01	< 0.02	< 0.02	

phospholipids were further analysed for ¹⁴C incorporation into individual fatty acids.

Hormonal treatments seemed to have only a slight effect on the conversion of linoleic acid into more polyunsaturated fatty acids by the testicular tissue in the immature hypophysectomized rats (Table 3), and the percentage of radioactivity incorporated into fatty acids of the ω -6 series was

not significantly altered. The 22:5 (ω -6) acid showed only slight radioactivity in spite of its high concentration and the high radioactivities in both linoleic acid and arachidonic acid. Significant incorporation was obtained in all the hormone-treated groups into a fatty acid tentatively identified as a 24:5 acid.

Incorporation of radioactivity into fatty acids

shorter than 18:2 represented breakdown of linoleic acid into short carbon units and their subsequent resynthesis into fatty acids of various chain lengths. The radioactivity in this fraction was higher in the FSH-treated group than in the other groups.

Hormonal therapy had little effect on the percentage fatty acid composition of the testicular phospholipids in the immature hypophysectomized rats, though there was a significant increase in the concentrations of a fatty acid, tentatively identified as 24:5, after both LH and testosterone treatment (Table 4). This acid, first reported by Davis *et al.* (1966), was detected in small but measurable quantities in the immature rat testis.

In hypophysectomized mature rats, the percentage of the ^{14}C radioactivity in the polyunsaturated fatty acids longer than 18:2 was lower than that in any one of the hormone-treated groups. FSH treatment raised these values only slightly. LH treatment caused a pronounced increase in the incorporation of radioactivity into polyunsaturated fatty acids of the $\omega-6$ family (20:4 and 22:5) (Table 5). The effect of TP was similar to that of LH, indicating that the LH effect may be mediated through testosterone. Little or no radioactivity could be found in the 24:5 acid in any of the groups. These hormonal influences on polyunsaturated fatty acid synthesis were reflected also in the con-

Table 4. *Effects of hormonal treatments on the percentage fatty acid composition of the phospholipids in the testicular tissue of hypophysectomized immature rats*

The results are expressed as percentages of the total fatty acids in phospholipids. Each result is the mean $\pm$ s.e.m. of values for a group of six animals. n.s., Not significant.

Fatty acid	Group ...	Fatty acid composition (% of total)			
		Control	FSH	LH	TP
16:0		29.2 $\pm$ 4.2	30.6 $\pm$ 4.0	26.2 $\pm$ 3.7	31.5 $\pm$ 4.2
18:0		7.1 $\pm$ 1.0	5.5 $\pm$ 1.1	10.6 $\pm$ 1.3	7.1 $\pm$ 1.1
18:1		20.7 $\pm$ 2.4	16.0 $\pm$ 3.0	17.6 $\pm$ 2.2	17.0 $\pm$ 1.9
18:2		5.4 $\pm$ 1.1	6.9 $\pm$ 1.1	5.2 $\pm$ 1.0	2.9 $\pm$ 0.5
20:4		15.7 $\pm$ 2.4	15.6 $\pm$ 2.6	14.8 $\pm$ 2.2	14.2 $\pm$ 1.9
22:4		1.7 $\pm$ 0.2	2.1 $\pm$ 0.5	Trace	1.6 $\pm$ 0.2
22:5		20.5 $\pm$ 3.2	21.6 $\pm$ 3.9	18.5 $\pm$ 2.4	18.9 $\pm$ 2.7
24:5		Trace	2.8 $\pm$ 0.5	6.8 $\pm$ 1.0	5.9 $\pm$ 1.0
<i>P</i> values for 24:5 acid					
		LH versus FSH		TP versus FSH	
		< 0.01		n.s.	

Table 5. *Effects of hormonal treatments on the incorporation of radioactivity from [1- ^{14}C]linoleic acid into various fatty acids in the phospholipid fraction of testes from hypophysectomized mature rats*

The results are expressed as percentages of the total radioactivity in the phospholipid fatty acids. Each result is the mean $\pm$ s.e.m. from six animals in each group. The radioactivities in 20:2, 20:3 and some unidentified components beyond 22:5 were 1% or less, and are not included in the Table. n.s., Not significant.

Fatty acid	Group ...	Radioactivity in fatty acid (% of total)			
		Control	FSH	LH	TP
Total < 18:2		31.5 $\pm$ 3.4	25.6 $\pm$ 4.8	25.0 $\pm$ 3.0	25.2 $\pm$ 5.2
18:2		62.3 $\pm$ 5.6	60.3 $\pm$ 5.6	52.4 $\pm$ 4.7	54.6 $\pm$ 5.0
20:4		3.2 $\pm$ 0.8	10.7 $\pm$ 1.1	14.4 $\pm$ 2.0	12.4 $\pm$ 2.0
22:5		2.0 $\pm$ 0.6	2.1 $\pm$ 0.7	5.2 $\pm$ 1.1	4.6 $\pm$ 1.1
Total > 18:2		5.9	14.7	22.7	20.6
<i>P</i> values					
		FSH versus			
		Control	LH versus FSH	TP versus FSH	
20:4		< 0.01	< 0.01	< 0.05	
22:5		n.s.	< 0.01	< 0.01	

Table 6. *Effects of hormonal treatments on the percentage fatty acid composition of the phospholipid fatty acids in the testicular tissue of testes from hypophysectomized mature rats*

The results are expressed as percentages of the total fatty acids in phospholipids. Each result is the mean $\pm$ S.E.M. of values for a group of six animals.

Fatty acid	Group ...	Fatty acid composition (% of total)			
		Control	FSH	LH	TP
16:0		47.5 $\pm$ 5.6	36.5 $\pm$ 5.1	36.1 $\pm$ 3.3	33.5 $\pm$ 4.6
16:1		1.6 $\pm$ 0.5	Trace	Trace	Trace
18:0		8.1 $\pm$ 1.1	10.7 $\pm$ 1.1	9.1 $\pm$ 1.0	7.3 $\pm$ 1.0
18:1		22.7 $\pm$ 2.4	20.5 $\pm$ 2.3	14.6 $\pm$ 1.6	12.2 $\pm$ 1.7
18:2		3.8 $\pm$ 1.0	6.0 $\pm$ 1.1	4.4 $\pm$ 1.0	5.1 $\pm$ 1.2
20:4		6.1 $\pm$ 1.0	10.3 $\pm$ 1.2	16.2 $\pm$ 2.3	16.4 $\pm$ 2.6
22:5		5.1 $\pm$ 1.2	9.0 $\pm$ 1.8	14.6 $\pm$ 3.2	18.2 $\pm$ 3.0
<i>P</i> values					
Fatty acid		FSH versus			TP versus FSH
		Control	LH versus FSH		
20:4		< 0.01	< 0.01		< 0.01
22:5		< 0.01	< 0.01		< 0.01

Table 7. *Percentage composition of fatty acids in total lipids of rabbit testes and ejaculated spermatozoa*

The results are expressed as percentages of the total fatty acids. Each result is the mean of three determinations.

Fatty acid	Fatty acid composition (% of total)	
	In testes	In ejaculated spermatozoa
16:0	37.1	35.9
18:0	5.3	3.1
18ald*	Trace	3.9
18:1	24.7	18.3
18:2	2.9	3.1
20:4	10.3	6.8
20:5	Trace	1.4
22 (?)†	Trace	8.5
22:5	14.1	17.3

* Stearaldehyde.

† Chain length characterized by hydrogenation; number or position of double bonds not determined.

Fig. 1. Appearance of radioactivity in the total lipids of semen fractions after [1-¹⁴C]linoleic acid had been given to rabbits. Δ , Radioactivity (counts/min.) in the seminal plasma of a single ejaculate; $\circ$, radioactivity (counts/min.) calculated for 10^8 sperms.

centrations of the fatty acids (Table 6). LH and TP treatments resulted in significant increases in the concentrations of 20:4 and 22:5 fatty acids, whereas FSH had a less marked effect.

Appearance of ¹⁴C in rabbit spermatozoa after the rabbits had been given [1-¹⁴C]linoleic acid. Table 7 shows the fatty acid composition of the total lipids in the testes and sperm of the rabbit. The fatty acid pattern in the sperm follows closely that of the

testes. This is expected because about 60–65% of the testicular tissue has been estimated to consist of spermatids and spermatozoa (Leblond, Steinberger & Roosen-Runge, 1963).

Fig. 1 shows the appearance of radioactivity in the total lipids in the sperm and the seminal plasma after the rabbits had been given 100 μ C of [1-¹⁴C]-linoleic acid. These experiments were done to

follow up a suggestion that the 22:5 (ω -6) fatty acid is incorporated into the sperm during the development of the spermatids (Davis *et al.* 1966). Attempts were made to determine the distribution of radioactivity in individual fatty acids, but, owing to the very low radioactivities in single ejaculates, these were not successful. It is assumed that most of the radioactivity was in linoleate and other polyunsaturated fatty acids. Radioactivity appeared in the seminal plasma within a few hours of the rabbits' being given [$1-^{14}\text{C}$]linoleic acid, and disappeared after about 10 days. Though the seminal plasma was not analysed further, this radioactivity was assumed to be mostly in the prostaglandins. Measurable amounts of radioactivity appeared in the sperm after about 5 days. The amount reached a peak at 14 days and then declined to a small amount after about 30 days. In about 60 days radioactivity had disappeared completely from the sperm. Allowing 9-10 days for the passage of the spermatozoa from the caput of the epididymis to the cauda (Orgebin-Crist, 1965), and assuming that no mixing of fresh and older spermatozoa took place in the cauda epididymidis, owing to the frequent ejaculations used in our experiments, these results might indicate an involvement of the polyunsaturated fatty acids in all stages of spermatogenesis.

DISCUSSION

The critical involvement of the polyunsaturated fatty acids in spermatogenesis and testicular development, first borne out by the classical experiment of Burr & Burr (1930), later gained experimental support from various workers (Finerty *et al.* 1957; Aaes-Jørgensen, Funch, Engel & Dam, 1956; Aaes-Jørgensen & Holman, 1958). Notable is a report by Noble & Carroll (1961) that erucic acid, a 22:1 acid, destroys the germinal epithelium in testicular tissue and induces permanent sterility. This result should be considered in relation to the reports of high concentrations of a 22:5 (ω -6) fatty acid in normal testes and its appearance during testicular maturation. Since erucic acid could conceivably compete with the 22:5 acid in forming structural elements, this might indicate a crucial role of the latter acid in normal testicular development.

In contrast with some earlier reports, the present study shows that the immature rat testis contains a significant amount of 22:5 (ω -6) fatty acid (15-20% of total fatty acids). The concentration is little affected 2 weeks after hypophysectomy or after subsequent treatment with gonadotrophic hormones, however. In contrast, hypophysectomy in mature rats causes a decline in the concentration of, and in the incorporation of ^{14}C radioactivity from

linoleic acid into, 22:5 acid, and also 20:4 acid, the results with LH-treated or TP-treated animals being used as a basis for comparison. The significance of these observations is not yet apparent. However, gonadotrophin preparations do not induce spermatogenesis in immature rats (Asdell, 1946), and the different response of the mature rat testes could be related to their spermatogenic activities, which may impose a particular demand on the polyunsaturated fatty acid pool. The possibility that these changes in ^{14}C incorporation might reflect changes in dilution of ^{14}C due to different sizes of free fatty acid pools or to different turnover rates of phospholipid fatty acids cannot, however, be excluded, and should be taken into consideration in the interpretation of these data.

The fact that the immature rat testis contains high percentages of arachidonic acid and docosapentaenoic acid 2-3 weeks after hypophysectomy indicates an involvement of these fatty acids with the spermatogonia or Sertoli cells, since these are the only structures reported to remain intact after hypophysectomy in the rat (Smith, 1939). Moreover, since ejaculated sperm in rabbits also contain an appreciable amount of arachidonic acid and docosapentaenoic acid, it is likely that these fatty acids are associated with spermatogenesis. Further evidence for this contention is provided by the report of Davis *et al.* (1966). The decrease in the concentrations of the arachidonic acid and docosapentaenoic acid in the hypophysectomized mature rat may then be explained by the arrest of spermatogenesis in hypophysectomized animals and its restoration by LH or testosterone therapy.

From their experiments with maturing rat testes, Davis *et al.* (1966) concluded that the docosapentaenoic acid is probably associated with the maturation of the spermatids. The observations in our experiments that in rabbits labelled sperms began to appear in the ejaculates 5 days after the rabbits were given [$1-^{14}\text{C}$]linoleic acid, and that hypophysectomized immature rat testes contained an appreciable amount of docosapentaenoic acid, would argue against their conclusions. However, we do not know into what fatty acids the label was incorporated in our studies, and it was not possible to determine the precise time at which the fatty acids appeared in the sperm lipids. It is noteworthy that the fatty acid composition of the rabbit sperm reported here is similar to that found for rat sperm by Dr J. G. Bieri (personal communication).

The stimulation of fatty acid incorporation into testicular tissue by FSH is probably a reflection of the general stimulation of growth of testicular tissue by FSH. The more pronounced effect on immature rat testis is presumably due to its increased sensitivity.

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REFERENCES

- Aaes-Jørgensen, E., Funch, J. P., Engel, P. F. & Dam, H. (1956). *Brit. J. Nutr.* **10**, 292.
- Aaes-Jørgensen, E. & Holman, R. T. (1958). *J. Nutr.* **65**, 633.
- Ahluwalia, B. & Holman, R. T. (1966). *Lipids*, **1**, 197.
- Asdell, S. A. (1946). *Patterns of Mammalian Reproduction*, p. 382. Ithaca, N.Y.: Comstock Publishing Co.
- Burr, G. O. & Burr, M. M. (1930). *J. biol. Chem.* **86**, 587.
- Cohen, P. P. (1957). In *Manometric Techniques and Tissue Metabolism*. Ed. by Umbreit, W. W., Burris, R. H. & Stauffer, J. F. Minneapolis: Burgess Publishing Co.
- Davis, J. T., Bridges, R. B. & Coniglio, J. G. (1966). *Biochem. J.* **98**, 342.
- Finerty, J. C., Klein, G. F. & Panos, T. C. (1957). *Anat. Rec.* **127**, 292.
- Folch, J. M., Lees, M. & Sloane-Stanley, G. H. (1957). *J. biol. Chem.* **226**, 497.
- Gambal, D. & Ackerman, R. J. (1967). *Endocrinology*, **80**, 231.
- Goswami, A. & Coniglio, J. G. (1966). *J. Nutr.* **89**, 210.
- Green, K. & Samuelsson, B. (1964). *J. Lipid Res.* **5**, 117.
- Kirschman, J. C. & Coniglio, J. G. (1961). *Arch. Biochem. Biophys.* **93**, 297.
- Leblond, C. P., Steinberger, E. & Roosen-Runge, E. C. (1963). *Mechanisms Concerned with Conception*, p. 1. New York: MacMillan.
- Noble, R. L. & Carroll, K. K. (1961). *Recent Progr. Hormone Res.* **17**, 97.
- Orgebin-Crist, M. C. (1965). *J. Reprod. Fertil.* **10**, 241.
- Panos, T. C. & Finerty, J. C. (1954). *J. Nutr.* **54**, 315.
- Smith, P. E. (1939). In *Sex and Internal Secretions*, 2nd ed., p. 931. Ed. by Allen, E., Danforth, C. H. & Doisy, E. A. Baltimore: Williams and Wilkins Co.