The Labelling of Ram Semen *in vivo* with Radioactive Phosphate and [*carboxy*-^{14}C]Stearic Acid

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(*Received* 2 *September* 1957)

Isotopes have been used on a number of occasions to study the rates of physiological processes within the male reproductive tract. Howard & Pelc (1950), Risley (1955) and Pelc (1957) used radioautography to investigate spermatogenesis in the testes after the injection of [^{32}P]phosphate or [8-^{14}C]adenine. Ortavant (1954*a*, *b*) determined the time for ^{32}P-labelled deoxyribonucleic acid to appear in the sperm at the head of the epididymis and also observed the time taken for the labelling of various parts of the epididymis. Heath, Rimington, Glover, Mann & Leone (1953) and later Heath, Rimington & Mann (1957) found that when [^{35}S]methionine was injected into the boar, maximum activity appeared in the seminal-plasma proteins of serially collected samples of semen within a few days, whereas there was a delay of at least 3 weeks before the sperm proteins with maximum labelling were ejaculated. Similarly, Bronsch & Leidl (1957) have recently found that it took 14 days for maximum ^{32}P activity to appear in ejaculated bull semen after the administration of labelled phosphate, and Sirlin & Edwards (1955) found that it required 28 days to elapse before radioactive sperm were ejaculated from mice injected with [8-^{14}C]adenine.

The present experiments were started in the first instance to gain information about the functions and origin of glycerylphosphorylcholine in the reproductive tract of the ram, by studying the rate at which it appeared labelled in the semen after the administration of radioactive phosphate. This choline-containing phosphodiester was first reported as a constituent of the seminal vesicles of the rat (Diament, Kahane & Lévy, 1952; Lundquist, 1953) and more recently a survey has shown it to be a universal constituent of mammalian semen, where it often occurs in considerable concentration (Dawson, Mann & White, 1957). Analysis of the epididymal seminal plasma of various farm animals showed this to be a rich source of glycerylphosphorylcholine, and indicated that some of the diester was formed in such parts of the reproductive tract as the testes or epididymides rather than in the accessory glands, where many of the seminal-plasma components are usually secreted.

The present isotopic results are consistent with the view that most of the seminal glycerylphosphorylcholine is formed in the epididymides. As the diester is probably produced by the hydrolysis of lecithin, experiments were made to determine whether its production was associated with a transfer of labelled fatty acids or lipid to the sperm; no evidence for such a transfer was found.

These isotopic experiments have been extended to include observations upon the rate of appearance of radioactivity in various phosphorus components of sperm in serially collected samples of semen after administration of ^{32}P. From these results assessments have been made of the time taken for sperm to pass through the epididymides and also for certain stages of spermatogenesis to occur in the testes. These measurements possess some advantage over methods used previously in that, if it can be assumed that the tracer causes no radiological damage to the testes, the observations have been made directly upon the sperm under normal physiological conditions.

EXPERIMENTAL

Collection and fractionation of semen

Rams (Clun Forest) were trained to use an artificial vagina and ejaculates were collected every 2 or 3 days during the breeding season. Lengths of bicycle inner tubing (1¾ in. diam.) were found to make very durable liners for the vagina and lubrication was provided with petroleum jelly except in experiments involving labelled stearic acid, in which no lubrication was used. The temperature was maintained at 40–42°, a 12v heating coil supplied through a mains transformer being used. After collection the sample of semen was stored at 37° or in a Thermos receiver. The volume was recorded, and small portions were used for motility determinations and sperm counts. The semen (0·5–1·4 ml.) was centrifuged and the seminal plasma (0·3–1·1 ml.) was removed with a teat pipette; the sperm were then washed four times with a calcium-free Ringer solution (Dawson *et al.* 1957) by centrifuging. In experiments requiring observations on the acid-soluble phosphorus of the sperm the last two washings were carried out with Ringer solution from which phosphate had been omitted.

Seminal plasma. The seminal plasma was introduced into 5 ml. of ethanol, and after mixing and standing for 5 min. the precipitate was removed by centrifuging. The supernatant was evaporated to dryness *in vacuo*, and the

residue was shaken with a mixture of 2 ml. of water and 3 ml. of a $CHCl_3$–butan-2-ol mixture (2:1, v/v). On centrifuging, the emulsion separated cleanly; the lower lipid–solvent layer contained insignificant amounts of lipid phosphorus. The upper aqueous layer, containing the glycerylphosphorylcholine, was evaporated to near dryness and applied as a short streak on a uni-dimensional filter-paper chromatogram. Development was carried out with a solvent which consisted of phenol saturated with water–ethanol (4:1, v/v); this enabled the diester, which ran almost with the solvent front, to be cleanly separated from the small amounts of other water-soluble phosphorus components of the seminal plasma present in the ethanolic extract. Location of the glycerylphosphorylcholine spots and their oxidation and solution before radioactive assay and phosphorus analyses have been described previously (Dawson, 1955).

Washed sperm. The washed sperm were treated with 10 ml. of ice-cold trichloroacetic acid soln. (10%). After standing at 0° for 10 min. the suspension was stirred and centrifuged. The supernatant was filtered and the radioactivity of the acid-soluble phosphorus was determined directly with a liquid counter, the phosphorus content being estimated in a 5 ml. portion of the solution. The precipitate was washed with 5 ml. of ice-cold water, and then treated with 10 ml. of a $CHCl_3$–methanol mixture (1:1, v/v) to extract the phospholipids. After standing for at least 1 hr., with occasional stirring, the mixture was centrifuged and the lipid extract was poured into a 100 ml. beaker containing 90 ml. of 0·01 M-$MgCl_2$ soln. After leaving for about 5–10 hr., the clear $CHCl_3$ lower layer containing the phospholipids was separated from the upper aqueous layer with a teat pipette. In this way any residual water-soluble phosphorus contaminating the phospholipids was eliminated (Folch, Ascoli, Lees, Meath & LeBaron, 1951). It was noticed that much cleaner interfaces of the $CHCl_3$ and aqueous layer could be obtained at this purification stage by drastically limiting the amount of petroleum jelly used to lubricate the artificial-vagina liner. The radioactivity of the lipid extract was estimated directly with a liquid counter after dilution to 10 ml. with ethanol. Phosphorus was determined in a 3 ml. portion after this had been evaporated to dryness and oxidized to inorganic phosphate.

The lipid-free sperm residue was washed with a further 10 ml. of the $CHCl_3$–methanol solvent, and then dried *in vacuo*. The residue was then incubated for 18 hr. at 37° with 3 ml. of N-NaOH soln., and after cooling to 0° the volume was brought up to 10 ml. by the addition of ice-cold 10% trichloroacetic acid soln. After standing for 10 min. at 0° the precipitated deoxyribonucleic acid was centrifuged off, washed with a further 10 ml. of the trichloroacetic acid soln., and then oxidized to inorganic phosphate. It was found that this could be very rapidly done by adding to it 0·05 ml. of 10% ammonium molybdate soln. and 1 ml. of 72% (w/w) $HClO_4$. On warming to 150° on an electrical digestion rack, shielded to avoid explosive risks, oxidation was very rapid. To avoid the formation of a water-insoluble precipitate the digestion mixture was removed from the rack immediately it turned pale yellow or became colourless. It was made up to 10 ml. with water and any turbidity removed by keeping it in a boiling-water bath until the precipitate dissolved. This solution, containing the deoxyribonucleic acid phosphorus, was assayed for radioactivity with a liquid counter, and phosphorus was determined in a 1 ml. portion. The supernatant from the precipitation of the deoxyribonucleic acid (the ribonucleic acid + phosphoprotein phosphorus fraction of Schmidt & Thannhauser, 1945) was also assayed for radioactivity and phosphorus. Its specific radioactivity seemed to be distinct from that of the other phosphorus-containing fractions of the sperm, and, although its chemical composition is uncertain and undoubtedly complex, it is for convenience designated as the 'residual phosphorus' fraction in this paper.

In some experiments the acid-soluble phosphorus of the sperm was not examined and here the Ringer-washed sperm were directly extracted with $CHCl_3$–methanol solvent to remove the lipids.

In the experiments involving sperm doubly labelled with [*carboxy*-^{14}C]stearic acid and [^{32}P]phosphate the collection and fractionation procedure was modified as follows. No lubrication was used on the liner of the artificial vagina, in order to avoid contamination of the semen with lubricant. The fatty acids and phosphorus in the seminal-plasma lipids were separated for radioactive assay in the following way. The $CHCl_3$–butan-2-ol-soluble substances present in the ethanolic extract of seminal plasma were recovered by removing the solvent *in vacuo*. The residue was then hydrolysed for 16 hr. at 100° with 2 ml. of 5 N-HCl. The fatty acids were extracted with 8 ml. of ether, and the ether solution was evaporated and eventually dried on a planchet in a desiccator ready for weighing and radioactive assay. The HCl hydrolysate was evaporated to dryness and oxidized; it was found to contain only negligible amounts of phosphorus and its radioactivity was not examined further. This process of separating the phospholipid phosphorus and lipid fatty acids was also repeated for the sperm lipids after they had been extracted and purified as described beforehand.

Administration and assay of isotopes, etc.

Labelled phosphate was administered with carrier (0·5 mg. of ^{31}P) in 5 ml. of isotonic NaCl soln. via the jugular vein. The dose used was approximately 1 mc/10 kg. At this level the animals showed no sign of radiation sickness, and the motility and morphological form of the ejaculated sperm appeared normal, so it is unlikely that any damage had been caused to the testes. [*carboxy*-^{14}C]Stearic acid (1 mc, 13 mg.) was also given via the jugular vein as a colloidal suspension in isotonic NaCl soln. Radioactivity of ^{32}P was measured in an M6 liquid counter and ^{14}C with a mica end-window counter. Specific radioactivity of the fatty acids was expressed as counts/mg., which was sufficiently accurate for the present experiments, and of the ^{32}P as counts/mg. of phosphorus. Decay corrections were applied to the ^{32}P observations. Phosphorus was determined by the method of Fiske & Subbarow (1925).

RESULTS

In initial experiments washed ram sperm were suspended in a Ringer solution containing labelled phosphate and fructose and incubated aerobically in Warburg vessels. No significant labelling of the sperm deoxyribonucleic acid or phospholipids occurred in 4 hr. even though respiration was

active. This provided a basis for performing experiments *in vivo*, for it seemed likely that in the reproductive tract the main labelling of these phosphorus components of the sperm would occur on their formation rather than by a continual dynamic exchange as is seen with tissue components. Immediately after the injection of labelled phosphate into a ram the specific radioactivity of the acid-soluble phosphorus in the blood plasma is very high, but its level then rapidly falls as the phosphate equilibrates with the phosphate of the bones and other tissues. Eventually the rate of decline levels out until a very slow and more or less constant decline is observed, which corresponds to the excretion of active phosphate by the animal and its replacement with the inactive phosphorus of the diet (Fig 1).

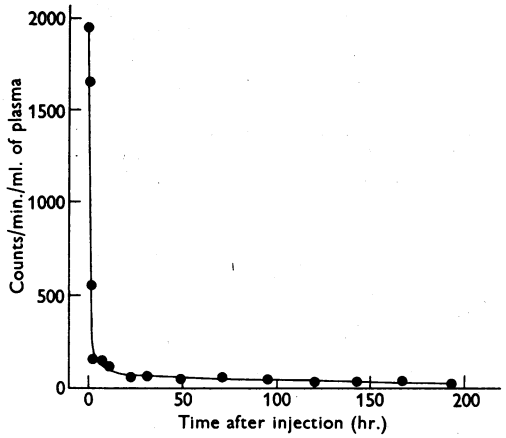

Fig. 1. The decline of radioactivity in the acid-soluble fraction of ram-blood plasma after the intravenous injection of labelled phosphate. The ram (wt. 94·6 kg.) was injected with 0·8 mc of [^{32}P]phosphate.

Isotope distribution in normal semen

When serial samples of semen were collected from rams which were ejaculating motile spermatozoa it was found that the various phosphorus fractions of the ejaculated semen became labelled in a definite sequence which was constant from animal to animal. In these experiments ejaculates were collected from the rams about two or three times a week, and it is probable that if the frequency of collection was varied the rate of appearance of labelled phosphorus components would also change. Fig. 2 shows a typical result obtained from the present experiments. At first, within a week of the administration of isotope to the animal, the acid-soluble phosphorus and the 'residual phosphorus' of the sperm acquired a small amount of labelling, but as collections were continued this declined again to a low level. From other observations to be described later it seems likely that this may be due to the initial secretion of very high specific-radioactivity phosphate by the accessory glands or contamination of the semen with urine phosphate of high activity. This highly radioactive phosphate in the seminal plasma might then enter or adhere to the sperm and introduce activity into the acid-soluble phosphorus fraction or exchange with the 'residual phosphorus' of the sperm. Then on the rapid decline of the specific activity of the blood-plasma phosphate the magnitude of this type of exchange labelling is likely to fall to a very low level in subsequent semen samples. Such an exchange of inorganic phosphorus has been observed with bull spermatozoa *in vitro*; when these were suspended in a saline containing labelled phosphate, radioactive ionic equilibrium was established within 5–45 min. (Bishop & Weinstock, 1948).

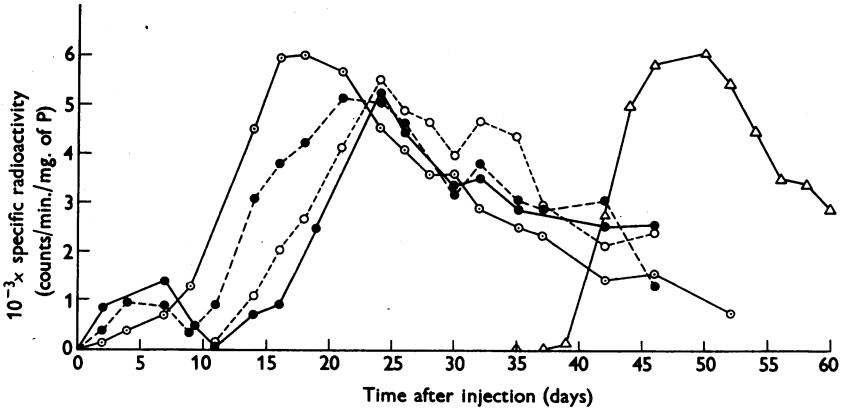

Fig. 2. Appearance of radioactivity in various phosphorus components of ram semen after the administration of labelled phosphate. ⊙—⊙, Seminal-plasma glycerylphosphorylcholine; ○- - -○, sperm phospholipid; ●- - -●, sperm acid-soluble phosphorus; △—△, sperm deoxyribonucleic acid; ●—●, sperm 'residual phosphorus.' The ram (wt. 41·5 kg.) was injected with 5 mc of [^{32}P]phosphate.

The labelling of the seminal-plasma glycerylphosphorylcholine began at a very low level in samples of semen collected a few days after the injection, but did not reach a maximum until about 2½ weeks later (Fig. 2). In four animals ejaculating motile sperm the maximum labelling of the glycerylphosphorylcholine occurred in ejaculates collected at 17, 18, 15 and 15 days after injection and always well before the phosphorus-containing components of sperm became maximally labelled. The maximum labelling of the sperm phospholipids occurred in four rams ejaculating motile sperm in ejaculates collected at 24, 21, 23 and 26 days after the isotope injection. Only one of these animals was examined for sperm acid-soluble phosphorus and 'residual phosphorus', and here as well as in another ram ejaculating non-motile sperm maximum labelling of these fractions occurred at about the same time as the sperm phospholipids.

No labelled sperm deoxyribonucleic acid appeared in the ejaculates until about 40–45 days after the administration of isotope: its specific radioactivity then increased with each successive ejaculation until a maximum was reached at 50 and 52 days in the two normal animals examined. Owing to the comparatively large amount of deoxyribonucleic acid in semen an accurate assessment of its specific radioactivity can be made after this length of time even though the half-life of ^{32}P is only 14·3 days. With other phosphorus components of the semen this is more difficult because of their lower total concentration, but nevertheless such readings as have been obtained gave no evidence of specific-radioactivity peaks corresponding with that of the deoxyribonucleic acid.

Isotope distribution in semen from a ram ejaculating non-motile sperm

In a single animal which normally ejaculated motile sperm, a transient period was experienced when the sperm ejaculated were non-motile but present at the same density as normally. Although the cause of this temporary defect could not be ascertained, isotope was administered during this period and a study made of the labelling of samples of semen collected serially. In nearly every respect this was similar to results obtained with normal semen except that the appearance of labelled glycerylphosphorylcholine was delayed (Fig. 3). Thus whereas in the four normal animals maximum labelling of the diester appeared at 15–18 days, and well before that of the sperm phospholipids, in the defective ram the maximum occurred at 30 days and almost simultaneously with the sperm phospholipids. While no general conclusions can be reached from this observation on a single ram it would appear that in this particular animal the secretion of radioactive glycerylphosphorylcholine in the reproductive tract or its passage to the exterior was considerably altered or delayed, although its concentration in the semen was normal. It is hoped that future opportunities may occur to confirm this finding with other animals.

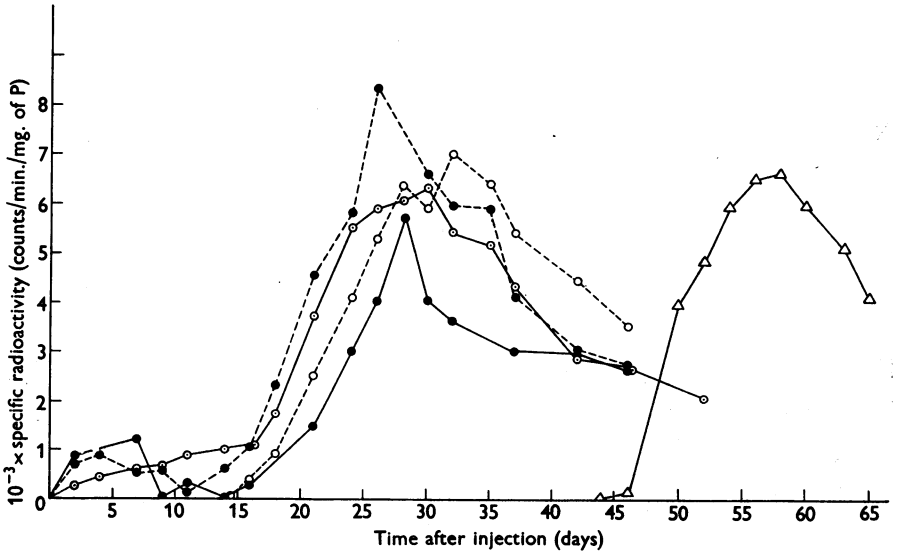

Fig. 3. Appearance of ^{32}P-radioactivity in various phosphorus components of semen in a ram producing non-motile sperm. ⊙—⊙, Seminal-plasma glycerylphosphorylcholine; ○- - -○, sperm phospholipid; ●- - -●, sperm acid-soluble phosphorus; △—△, sperm deoxyribonucleic acid; ●—●, sperm residual phosphorus. The ram (wt. 65·5 kg.) was injected with 7·5 mc of [^{32}P]phosphate.

Effect of severing the connexion between testes and epididymides

In order to obtain more information about the ^{32}P-labelling of semen other than in the testes, in one animal the connexions between the testes and epididymides were surgically severed. On one side the entire testis was removed and the epididymis with its blood supply was left intact. On the other side the testis was left *in situ*, to supply testosterone, and the head of the epididymis was removed. Three hours after the operation the animal was injected with [^{32}P]phosphate and serial samples of semen were then collected as usual. Considerable swelling of the scrotum occurred initially, and slight fever, but nevertheless collections of semen every 2–3 days were successfully carried out. Fig. 4 shows the total sperm present in the ejaculated semen, from which it can be concluded that it took approx. 15 days for sperm to be virtually cleared from the epididymis. For the first three collections the sperm were motile but then, possibly because of the transient slight fever of the animal, they became progressively less motile. The sperm phosphorus-containing components showed little labelling except in the acid-soluble phosphorus fraction. Part of this labelling may be due to the presence of active glycerylphosphorylcholine in or on the sperm, as they are known to contain small quantities of the diester which is not removed by washing; some may also arise from an exchange with highly active accessory-gland secretion or urine phosphate after ejaculation. No significant labelling of sperm phospholipids, deoxyribonucleic acid or 'residual phosphorus' occurred in these sperm obtained from the epididymides.

The decrease in the number of sperm ejaculated was associated with a fall in the concentration of glycerylphosphorylcholine. Nevertheless the specific-radioactivity curve of the diester was very similar to the normal pattern, which would suggest that part at least of the diester has an extratesticular origin.

Labelling of semen after simultaneous administration of [^{32}P]*phosphate and* [carboxy-^{14}C]*stearic acid*

In order to find whether any correlation existed between the formation of glycerylphosphorylcholine and the labelling of the fatty acids present in semen, an experiment was performed in which a ram was injected simultaneously with both labelled phosphate and [*carboxy*-^{14}C]stearic acid. The appearance of labelling of the phosphorus-containing components of the ejaculated semen followed a course which was typical (Fig. 5). The first ^{14}C-labelling of the sperm fatty acids appeared in the ejaculate soon after injection, i.e. within

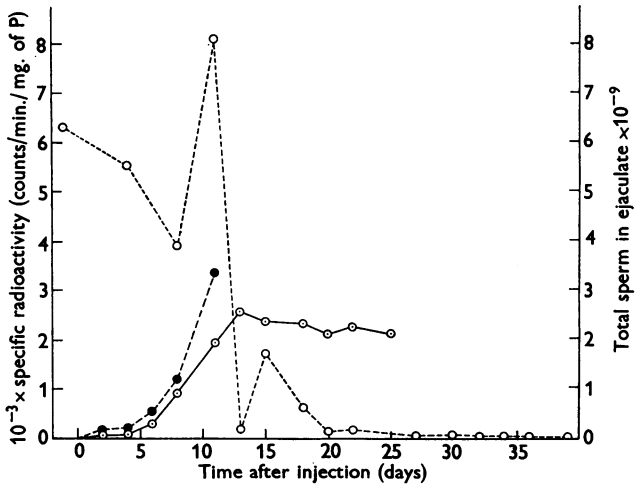

Fig. 4

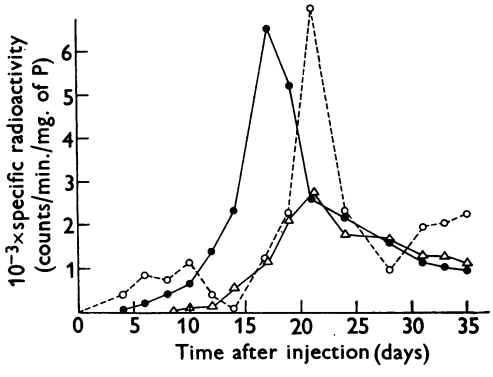

Fig. 5

Fig. 4. Radioactivity (^{32}P) and number of sperm in the semen of a ram in which the connexions between the testes and epididymides had been surgically removed. ○- - -○, Total sperm in ejaculate; ⊙—⊙, seminal-plasma glycerylphosphorylcholine; ●- - -●, sperm acid-soluble ^{32}P. The ram (wt. 74 kg.) was injected with 2·7 mc of [^{32}P]phosphate.

Fig. 5. Specific radioactivity of the semen glycerylphosphorylcholine (^{32}P), phospholipids (^{32}P) and sperm fatty acids (^{14}C) after the injection of labelled phosphate and [*carboxy*-^{14}C]stearic acid. ●—●, Seminal-plasma glycerylphosphorylcholine (^{32}P); △—△, sperm phospholipid (^{32}P); ○- - -○, sperm fatty acid (^{14}C). The ram (wt. 56·6 kg.) was injected with 3·5 mc of [^{32}P]phosphate and 1 mc of [*carboxy*-^{14}C]stearic acid.

10 days, and this suggests an accessory-gland origin. In this connexion maximum ^{14}C-labelling of the seminal-plasma fatty acids was found to occur soon after the injection of the isotope and Bishop & Lovelock have recently obtained evidence that isolated bull sperm can incorporate added [*methyl*-^{14}C]acetate into their lipid fatty acids (Austin & Bishop, 1957 and personal communication). There was no peak in the sperm fatty acids corresponding to the time when maximum ^{32}P-activity was found in the ejaculated glycerylphosphorylcholine, but as is to be expected a distinct ^{14}C peak appears in them at the time of maximum labelling of the sperm phospholipids.

DISCUSSION

It has been found that when a ram is injected with labelled phosphate the specific radioactivity of the acid-soluble phosphorus in the blood plasma rapidly declines as the isotope equilibrates with the phosphorus in the tissues. While there is some evidence that the passage of phosphate ions from the blood plasma into the testes may be slower than with other organs (Hevesy & Ottesen, 1943), it is to be expected that once inside the tissues of the reproductive tract the labelled phosphorus atoms will rapidly be incorporated into the metabolism which is responsible for the synthesis of the various phosphorus-containing components of the sperm and seminal plasma. This will mean that comparatively soon after the injection the various phosphorus-containing components used in the formation of the sperm and seminal plasma will appear in the reproductive tract in a radioactive form. Thus any delay in the appearance of the labelled component in the ejaculated semen must largely represent the time taken by various physiological processes in the reproductive tract before that component is ejaculated. Such processes would include, for example, the completion of spermatogenesis or the passage of sperm through the much convoluted tubule of the epididymis. This argument would only be valid if no appreciable exchange of the phosphorus in the seminal phosphorus compounds occurs after their formation. Experiments *in vitro* have indicated that this is probably true for the structural elements, e.g. phospholipids and deoxyribonucleic acid of ejaculated sperm, but it is apparent that some exchange of the acid-soluble phosphorus and 'residual phosphorus' fractions does occur, possibly by a passage of high-activity phosphate into the sperm from the seminal plasma after ejaculation.

One experimental point which has been clearly established in the present work is that, in the ram ejaculating motile sperm, labelled glycerylphosphorylcholine appears in the semen appreciably before the phosphorus components of the sperm become labelled. Previous work has indicated that in many animals glycerylphosphorylcholine is present in the epididymal seminal plasma at a considerable concentration (Dawson *et al.* 1957), which would suggest that it is formed either in the testis or epididymis. If then the glycerylphosphorylcholine which appears as a component of the plasma in ejaculated semen moves through the epididymis at an equal or slower rate to that of the sperm the present results would indicate that a considerable formation of the phosphodiester occurs in the epididymis. This is supported by the observation that considerable ^{32}P-labelling of seminal-plasma glycerylphosphorylcholine occurred in an animal surgically treated to sever the connexion between the testis and epididymis. However, a puzzling anomaly does remain: in one animal ejaculating non-motile sperm the labelling of the glycerylphosphorylcholine of the semen appeared at a point which exactly corresponded to the labelling of the phosphorus component of the sperm apart from deoxyribonucleic acid. This cannot be due to any reduction in the rate of secretion of glycerylphosphorylcholine into the semen because the amounts ejaculated by the animal were normal.

No explanation has yet been found for the considerable quantities of glycerylphosphorylcholine found in epididymal seminal plasma. So far the only metabolic pathway shown for the formation of glycerylphosphorylcholine in mammalian tissues appears to be by the hydrolysis of lecithin (e.g. Dawson, 1955). One possibility would be that in the reproductive tract lecithin is broken down in this way, and that the fatty acids are donated to the sperm in the form of some lipid, the glycerylphosphorylcholine formed in the process passing out as a by-product. In this connexion it was suggested by Redenz (1924) from histological observations that the epididymis was responsible for the formation of the lipid capsule of the sperm. However, in an experiment in which labelled stearic acid was administered simultaneously with labelled phosphate to a ram, no evidence of a transfer of this type could be detected; at the time of the appearance of ^{32}P-labelled glycerylphosphorylcholine in the semen no equivalent ^{14}C-labelling peak was observed in the fatty acids of the sperm. On the other hand, as is to be expected, an equivalent ^{14}C peak was clearly observed at the time of the maximum ^{32}P-labelling of the sperm phospholipids.

Another point which has been established is that after injection of [^{32}P]phosphate in the ram, the deoxyribonucleic acid of the sperm appears labelled in serially collected ejaculates at a con-

siderable time after the other phosphorus components of the sperm are ejaculated in a radioactive form. One explanation of this would be that the other phosphorus components become labelled during the passage of the sperm down the reproductive tract some weeks after they have left the testes. Ortavant (1954*a*), for example, found some evidence for an exchange of ^{32}P of this type at the head of the epididymis. However, from histological observations it is apparent that the sperm is virtually morphologically complete as it leaves the testes and one would expect the maximum labelling of, for example, the sperm phospholipids to occur during the formation of the sperm structure in the testes. Moreover, when the passage of the sperm from the testes was suppressed surgically the sperm in the epididymis did not become significantly labelled with ^{32}P except in their acid-soluble phosphorus fraction. If then the phosphorus components of the sperm become labelled mainly in the testes this must mean that either labelled deoxyribonucleic acid for sperm construction is formed considerably after, for example, labelled phospholipid, or alternatively that both are formed at the same time and that the nucleic acid is used in the formation of the sperm at a time during spermatogenesis well before that of the phospholipids. The whole concept of dynamic turnover of tissue components is against the first idea, for however enormous the pool size of the precursor of sperm deoxyribonucleic acid one would expect some labelling of this to occur even though it might be small compared with that of the phospholipids. In rats, for example, Hevesy & Ottesen (1943) found that at least 2·6% of the testicular deoxyribonucleic acid was replaced daily by newly synthesized material, and Pelc (1957) found that up to 12% of the spermatogonia possessed labelled deoxyribonucleic acid in their nuclei 1 day after the injection of [8-^{14}C]adenine. It seems much more likely therefore that deoxyribonucleic acid is formed during spermatogenesis only during the mitotic divisions taking place during the formation of the spermatocytes, whereas it is likely that phospholipid would be added up to the completion of the finished sperm. Evidence is accumulating from spectrophotometric examination of cells that the primary spermatocyte contains all the deoxyribonucleic acid necessary for the formation of the nuclei of the four spermatozoa which it finally produces (Lison & Pasteels, 1949; Swift, 1950; Schrader & Leuchtenberger, 1950). Furthermore, experiments with [8-^{14}C]adenine in the mouse testis have shown heavy incorporation into the deoxyribonucleic acid of non-dividing spermatogonia and primary spermatocytes, a small incorporation into secondary spermatocytes and a very small incorporation in the later stages of spermatogenesis (Pelc, 1957). If the production of spermatozoa is a continual process this would mean that sperm with labelled phospholipids would pass from the testis some time before those with labelled deoxyribonucleic acid. The time elapsing therefore between the first appearance of labelled phospholipid in the sperm of the ejaculate and labelled deoxyribonucleic acid (25–35 days) would represent the minimum time which it takes for the primary spermatocyte to be converted into the spermatozoa during spermatogenesis. In experiments with rams injected with [^{32}P]phosphate Ortavant (1954*b*) found that it took approximately 30 days for sperm labelled in their deoxyribonucleic acid fraction to appear at the head of the epididymis. This time therefore agrees well with the interpretation placed on the present results.

The present experimental evidence suggests that no appreciable ^{32}P exchange of sperm phospholipids occurs after they have left the testes. If this is so the results can be used to derive the minimum time necessary for spermatozoa to pass through the epididymides. Thus in four animals which were ejaculating naturally three times a week it took 13, 14, 10 and 13 days for labelled phospholipids to appear in the sperm. It is clear, however, that the rate of passage of sperm through the epididymis will depend to some extent upon the frequency of ejaculation and other biological factors. Thus by killing, at various intervals, rams which had been injected with [^{32}P]phosphate and examining the epididymides for activity, Ortavant (1954*a*) calculated that it took 20 days for sperm to pass through the epididymides in animals not sexually active.

The advantage of using isotopic labelling of the sperm to make these measurements is that provided no radiation damage is done to the testes, the movement of the sperm is assessed directly under normal physiological conditions. Previous estimates have usually relied on the injection of indian-ink particles into the testes, and it is apparent that such foreign bodies may not move at the same rate as the sperm themselves. Gunn (1936) found that when indian-ink particles were injected into the testes of rams it took 11 days in sexually resting rams, and 5 days in animals frequently caused to ejaculate by electrical stimulation, for the particles to pass through the reproductive tract. On the other hand, McKenzie & Phillips (1934) found that after scrotal insulation to cause heat degeneration of the testes it took an average of 8·8 days (4–13) for abnormal sperm to pass through the genital passages of rams copulating at the rate of approximately once a day. In arriving at this result they assume that no morphological change is brought about in the sperm by the heat after the completion of spermatogenesis.

SUMMARY

1. Rams were injected with [^{32}P]phosphate and [*carboxy*-^{14}C]stearic acid, and a study was made of the rate of appearance of labelled phosphorus compounds and fatty acids in the seminal plasma and sperm of serially collected samples of semen.

2. The maximum ^{32}P-labelling of seminal glycerylphosphorylcholine occurred some 15–18 days after injection, and next appeared the sperm phospholipids, acid-soluble phosphorus and a fraction designated as residual phosphorus, all having maxima at about 21–26 days. The sperm deoxyribonucleic acid appeared in a labelled form much later, reaching a maximum at 50–52 days.

3. In one animal ejaculating non-motile sperm the appearance of labelled glycerylphosphorylcholine in the semen was considerably delayed compared with normal rams.

4. In a ram in which the connexions between the epididymides and testes were severed surgically the seminal-plasma glycerylphosphorylcholine and sperm acid-soluble phosphorus became labelled with ^{32}P, but no appreciable activity appeared in the phospholipids, residual phosphorus and deoxyribonucleic acids of the ejaculated sperm.

5. Simultaneous injection of a ram with [^{32}P]-phosphate and [^{14}C]stearic acid did not indicate any transfer of labelled fatty acids to the sperm at the time of formation of glycerylphosphorylcholine.

6. From these results with isotopes an assessment has been made of the minimum time taken for the sperm to pass through the epididymides and also the time taken for the spermatocytes to be converted into the spermatozoa during spermatogenesis in the ram testes.

The author is grateful for the valuable advice received and interest displayed by Sir Rudolph Peters, F.R.S., and Dr T. Mann, F.R.S., during this investigation. He is indebted to J. Clark and D. Wise, who have assisted in the collection of semen. Thanks are given to Mr R. W. White who has been responsible for the histological examination of semen and to Miss N. Hemington who has given valuable assistance in the laboratory. Thanks are also extended to Mr K. J. Hill, who was responsible for the surgery involved, and Dr D. Lewis who kindly undertook the intravenous injection of isotopes.

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Comparative Studies on the Liver Sulphatases

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(*Received* 12 *August* 1957)

Although the widespread distribution of the arylsulphatases is well known, there is little information available on the amounts of these enzymes present in the various species, the only attempt at comparative studies being that of Rutenburg & Seligman (1956) whose work is diminished in value because of the failure to appreciate the occurrence of several different sulphatases in their preparations. At least three arylsulphatases occur in mammalian tissues; sulphatases *A* and *B* (Roy, 1953*b*, 1954*a*) rapidly hydrolyse dipotassium 2-hydroxy-5-nitrophenyl sulphate (nitrocatechol sulphate, NCS) but attack potassium *p*-nitrophenyl sulphate (NPS) only very slowly. These two enzymes may be separated by paper electrophoresis at pH 7, sulphatase *A* moving towards the anode