in light petroleum, contained over 50 % of non-lipid impurities. A large part of this impurity was readily removed by the method of Folch *et al.* (1951) with only small accompanying loss of lipids. This loss was, however, selective. Some additional non-lipid was removed with an ion-exchange resin.

2. The purified extract was examined by counter-current distribution between light petroleum and 85 % ethanol. The component lipid classes found were lecithin, phosphatidylethanolamine, plasmalogens, waxes, hydrocarbons, inositol lipids and unidentified material. This last included possibly three different types of lipid.

3. The plasmalogen could not be satisfactorily characterized and much of it probably did not correspond to the established formula. The inositol

lipids included two and possibly three components. Certain unidentified lipids were accompanied by a nitrogenous base previously reported (Olley & Lovern, 1953) and another fraction by a different nitrogenous compound or compounds.

4. The lecithin contained a fatty acid mixture generally similar to that of other lecithins previously isolated from haddock flesh. The unidentified lipids contained fatty acids showing certain variations from the lecithin pattern, but all similar in containing 20–30 % of saturated components and having C_{20} acids, arachidonic acid or an isomer, as the most plentiful constituent.

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Zinc and Carbonic Anhydrase in Human Semen

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The prostate glands of the rabbit and of man, and the dorsolateral prostate of the rat, contain a very high concentration of zinc (Mawson & Fischer, 1951, 1952*a*). The source of the zinc found in human semen by Bertrand & Vladesco (1921) and in ram sperm and seminal plasma by Mann (1945) is not known, but it seemed possible that this could be investigated in man by collecting the first, middle and last portions of each ejaculate separately and by estimating the zinc content of the separate fractions. Gutman & Gutman (1941) showed that acid phos-

phatase, which can be assumed to originate in the prostate gland, occurs mainly in the first fraction. Lundquist (1949) and Pryde (1950) estimated fructose in separate fractions, and found most of the fructose in the last fraction which they assumed to come from the seminal vesicles.

Any attempt to explain the presence of zinc in tissues must take into account the fact established by Keilin & Mann (1940) that zinc is the prosthetic metal of carbonic anhydrase. Mawson & Fischer (1952*b*) have shown that the high concentration of

zinc in rat dorsolateral prostate is accompanied by high carbonic anhydrase activity, though only part of the zinc in that tissue could be accounted for as carbonic anhydrase. A few estimations of carbonic anhydrase have been made on human sperm and on the seminal plasma obtained from three separated fractions of the semen, but only small amounts of the enzyme were found in the sperm, and little or none in the seminal plasma.

METHODS

Glassware. All apparatus used for zinc analyses was cleaned with 2N-HNO₃ overnight, and was then washed in zinc-free distilled water. Pyrex glassware was used throughout, and all vessels were kept covered except when in use.

Collection of specimens. Ejaculates from five donors were collected in three fractions, in three separate containers. The proportions of fractions varied, but usually the second fraction was the largest. The semen was allowed to liquefy by standing at 37° for 15–30 min. and samples were removed for morphological examination and sperm count. The specimens were then centrifuged, the plasma removed as completely as possible, and the sperm from the three fractions from each donor pooled and recentrifuged. The spermatozoa were not washed, as it was feared that this might lead to loss of zinc.

Analytical methods. Samples of sperm and plasma were measured into platinum dishes, dried overnight at 95–105° and ashed for 16 hr. at 520°. Zinc was estimated in the ash by the method of Vallee & Gibson (1948). Carbonic anhydrase was estimated by the colorimetric method of Roughton & Booth (1946) in sperm which had been repeatedly frozen and thawed and made up to volume in saponin solution (1.0 mg./100 ml.). The buffer used was 0.022M veronal in 0.022M sodium veronal. (A misprint in the original paper gives the sodium veronal concentration as 0.22M.) Our unit was defined as the amount of enzyme which would double the rate of the uncatalysed reaction under our working conditions, and it was found that one unit of purified enzyme (0.22% Zn) had a dry weight of 0.225 µg. Our unit has about one-half the value of that used by Keilin & Mann (1940) who carried out their estimations by the manometric method of Meldrum & Roughton (1933).

RESULTS

Characteristics of semen fractions. Specimens were obtained from each donor on three occasions and zinc estimations were made on the whole semen, the plasma and the sperm. The extent to which the fractions differed from one another may be seen in Table 1; sperm counts in the three fractions differed significantly from each other ($P < 0.01$), whereas the content of dry matter was about the same in all fractions. The results from one donor (no. 4) have been omitted in calculating the mean dry weights because the specimens were very small (total volume about 1 ml.) and had dry weights averaging 5.1, 4.6 and 5.9% respectively for fractions 1, 2 and 3. A high proportion of the sperm from donor no. 1

Table 1. *Sperm count and content of dry matter in fractionated semen*

(Number of specimens: 12 for fractions 1 and 2, 11 for fraction 3. Results are means ($\pm$ S.E. for sperm count).)

Fraction no.	Sperm count (millions/ml.)	Dry matter (g./100 ml.)
1	320 $\pm$ 55.3	7.8
2	73 $\pm$ 16.4	8.3
3	20 $\pm$ 4.3	8.5
Separated sperm		17.6

were abnormal in form, and the semen of donor no. 2 liquefied only with difficulty, though rapid liquefaction occurred if a little of the plasma from another donor was added.

The appearance of the semen when viewed with the phase-contrast microscope showed consistent differences between the three fractions in all cases. The sperm in the first fraction, which contained about 80% of the total number of cells, were highly active, whereas those in the second portion were nearly all sluggish or dead. In the third portion some feebly motile sperm were seen, but most of those present were dead. A very few highly motile sperm did, however, occur in the third specimen, and it was shown that the plasma of the second and third fractions was not toxic, because it had no detrimental effect on the motility of sperm taken from fraction 1.

Zinc in semen. The zinc content of the three fractions of semen is shown in Table 2, together with that of the pooled sperm. The zinc content of the plasma separated from each fraction is not shown because it did not differ significantly from that of the uncentrifuged specimen.

The figures for sperm are not complete, owing to occasional difficulty in separation of sperm from mucous material or because insufficient sperm were available for weighing with the necessary accuracy. The statistical analysis in the case of the sperm has therefore been based upon five figures, each of which is the mean value obtained from one donor. The zinc content of the first fraction of semen differs significantly from that of the second ($P < 0.01$), but the second and third fractions are not statistically distinguishable in this respect ($0.2 > P > 0.1$).

Dialysis of seminal plasma. Four specimens of unfractionated semen were centrifuged and the plasma dialysed overnight in cellophane bags, using running distilled water at 4–6°. The zinc content decreased in all specimens, but Table 3 shows that the proportion of dialysable zinc was variable.

Carbonic anhydrase in semen. The seminal plasma contained less than 0.005 unit carbonic anhydrase/mg., which is a negligible amount, and although the enzyme was present in the sperm, the concentration was no greater than that found in perfused brain or

Table 2. *Zinc content of semen fractions and of separated sperm*

Donor no.	Zinc content in fractions (mg. Zn/g. dry wt.)			
	1	2	3	Centrifuged pooled sperm
1	2.67	0.70	0.19	2.34
	3.54	0.60	0.25	1.56
	2.19	0.71	0.18	3.91
2	5.98	2.27	2.22	—
	4.61	2.74	1.45	2.00
	3.12	2.86	2.11	1.32
3	1.60	0.32	0.43	—
	1.99	0.49	0.25	1.25
	1.59	0.49	0.33	—
4	3.58	1.63	—	—
	2.81	1.81	1.44	—
	3.81	2.97	2.14	3.55
5	1.93	1.16	0.41	0.94
	2.60	1.39	0.81	0.94
	1.92	0.80	0.47	0.77
Mean	2.93	1.40	0.91	1.99
No. of specimens	15	15	14	5
S.E.	0.316	0.241	0.212	0.483

Table 3. *Effect of dialysis on zinc content of seminal plasma*

(Values corrected for change in volume during dialysis.)

Specimen	Zn content ($\mu\text{g./ml.}$)		
	Before dialysis	After dialysis	% dialysable
A	81.3	69.4	15
B	137.0	98.6	28
C	216.3	54.0	75
D	101.7	38.0	63

testis. Four specimens of human sperm had a mean carbonic anhydrase content of 0.24 unit/mg. (range, 0.19–0.31; S.E., 0.031); the average figure for human erythrocytes is 5.1 units/mg. Meldrum & Roughton (1933), using a unit which is about ten times as great as the one used in our work, obtained a value for carbonic anhydrase in rabbit sperm which is even lower than the one we obtained in human sperm.

DISCUSSION

The values obtained by Bertrand & Vladesco (1921) for zinc in three specimens of human semen were 53, 152 and 220 $\mu\text{g./ml.}$ or 572, 1152 and 2020 $\mu\text{g. zinc/g. dry matter.}$ These figures, together with analyses of semen and male genital organs of several different species, led them to suggest that 'zinc plays a part in reproduction and also in the regulation of internal secretions'. Our results have confirmed and extended the observations of Bertrand & Vladesco. Mann (1945), using ram semen, found a zinc content of 9.80 $\mu\text{g./ml.}$, of which 7.80 $\mu\text{g.}$ was in the sperm. Ram semen contains about 50% (v/v), sperm, so if we assume a dry weight of 20% (w/w), for the sperm and

8% (w/v), for the plasma, the zinc content of both sperm and plasma was within the range 50–80 $\mu\text{g./g. dry weight.}$ These are less than one-tenth the values found for human semen, but ram semen resembles that of man in the approximate equality of the zinc concentration in sperm and in plasma when expressed on a dry-weight basis.

In order to appreciate the very high concentration of zinc in human sperm it is necessary to compare it with values obtained for other tissues. Table 4 contains our own figures for human sperm and prostate, and for a series of rat tissues, and also some results obtained by Eggleton (1940) from analyses of human tissues. It can be seen that human sperm contains more zinc than any other

Table 4. *Zinc content of human sperm compared with that of other human and rat tissues*

(Values for human tissues taken from Eggleton (1940) except for prostate and sperm (this work).)

Tissue	Mean Zn content ($\mu\text{g./g. dry wt.}$)	
	Human	Rat
Brain	43*	70
Lung	67	87
Spleen	72	86
Heart	100	83
Pancreas	135	101
Kidney	186	106
Muscle	226	50
Liver	245	107
Testis	62	212
Epididymis	—	216
Prostate	859	891†
Sperm	1998	—

* Cerebrum.

† Dorsolateral prostate.

human tissue so far examined. In the rat, the highest concentration of zinc occurs in the lateral portions of the dorsolateral prostate, namely 2900 $\mu\text{g. zinc/g. dry weight}$. Our experiments show that the first fraction of semen contains most of the sperm in the total ejaculate, even when the volume of semen is less than 1 ml. We have assumed that the first fraction was a mixture of epididymal and prostatic secretion and the second fraction was a mixture of prostatic and vesicular secretions. The third fraction may be assumed to originate mainly in the seminal vesicles. Human semen contains stringy mucous material which is difficult to separate from centrifuged sperm. Much of this contaminant may be avoided by using sperm from the first semen fraction only, and most of the remainder may be removed by centrifugation for 5 min. at 1000 rev./min. if the fraction is first diluted with physiological saline containing 0.2% glucose and incubated at 37° for 30 min. The residual sperm is free from 'stringy' material and contains about the same amount of zinc as unwashed sperm.

Although the sperm contain a high concentration of zinc their relative volume is so small that it was not possible to show any significant difference between the zinc content of whole semen and that of the seminal plasma. Human prostate contains a very large amount of zinc (Table 4), so it is not surprising that there is a high concentration of zinc in the first fraction of the semen. It is noteworthy, however, that the amount in the secretion is even higher than that in the gland itself, and that part of the zinc is in a dialysable form. The function of the prostatic secretion, apart from the fact that it dilutes the semen, is obscure, but it now appears that it contributes a large amount of zinc to the medium in which the sperm, themselves rich in zinc, will be suspended. It must also be remembered that, as Mann (1945) has pointed out, the seminal plasma is not merely an inert vehicle, since it has a metabolism of its own.

Very little is known about the functions of zinc in living matter apart from its occurrence as the prosthetic metal in certain enzymes, particularly carbonic anhydrase. Recent work by Foster & Denison (1950), Nason, Kaplan & Colowick (1951), Nason, Oldewurtel & Propst (1952), Quinlan-Watson (1951, 1953) and Robinson (1951) has shown that zinc deficiency during the growth of

Rhizopus nigricans, *Neurospora crassa*, *Aspergillus niger*, oats, clover and tomatoes may lead to gross disturbances in the enzyme patterns of these organisms. Failure to synthesize pyruvic decarboxylase, alcohol dehydrogenase, aldolase, and the enzyme which forms tryptophan from serine and indole, and also abnormally high production of certain oxidases and of diphosphopyridinenucleotidase have been reported. The suggestion that zinc is in some way essential for the synthesis of certain enzymes, of which it is not a prosthetic metal, may have a bearing on the presence of zinc in sperm, which possess a particularly active metabolism. It is also of interest in connexion with our observation (Mawson & Fischer, 1952c) that there is a high concentration of zinc in the Paneth cells of the small intestine of the rat. These cells are generally regarded as the site of synthesis of the digestive enzymes of the small intestine.

The amount of carbonic anhydrase in the sperm cannot account for more than a minute proportion of their zinc content. About 30% of the zinc in human erythrocytes occurs as part of the carbonic anhydrase molecule, and about 2.5% of the zinc in rat lateral prostate may be accounted for in this way, but in the case of human sperm only about 0.05% of the zinc can occur in combination with carbonic anhydrase.

SUMMARY

1. Zinc occurs in high concentration in human semen.
2. The highest concentration of zinc is found in the first fraction of the ejaculate, which is mainly composed of prostatic secretion.
3. Spermatozoa contain a higher concentration of zinc than any other human tissue yet examined.
4. The zinc contained in seminal plasma is partly in a dialysable form.
5. Seminal plasma contains negligible amounts of carbonic anhydrase. A little carbonic anhydrase is found in spermatozoa, but only about 0.05% of the zinc present in the sperm can be accounted for in this way.

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The Amino Acids of Cytoplasmic and Chloroplasmic Proteins of Barley

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During the germination and the early phases of development of barley plants there is a rapid synthesis of protoplasmic proteins, particularly in the young leaves and root system of the seedlings (Folkes, Willis & Yemm, 1952). These proteins form an integral part of the protoplasts of the cells, and ultimately most of the nitrogen assimilated by the plant is incorporated in cytoplasmic and chloroplasmic structures. The data reported in this paper were collected in the course of an investigation into the relation between the synthesis of the protoplasmic proteins and the metabolism of amino acids in the young seedling.

Extensive data have been published on the protoplasmic proteins of various plants and have been reviewed by Chibnall (1939) and Lugg (1949). Yemm (1950) gave an account of the separation and partial analysis of preparations containing a mixture of chloroplasmic and cytoplasmic proteins from mature leaves of barley. This work has now been extended to a similar preparation from barley seedlings and to a preparation from mature leaves which consists mainly of cytoplasmic components. Moreover, in the present analyses a more comprehensive investigation of the constituent amino acids in these preparations has been made; analytical data, obtained chiefly by microbiological assay, are recorded for eighteen amino acids.

A short account of the work has already been given to the Biochemical Society (Yemm, 1951; Folkes & Yemm, 1951).

METHODS

Preparation of proteins

Mixed protoplasmic proteins of seedlings. Seedlings of barley were grown by soaking in water overnight 500 g. of grain (N, 2.5% of dry weight) and sowing in moist sand in a cold frame at about 15°. After 14 days the seedlings were uprooted, washed free from sand, and squeezed fairly dry. All the seedlings (total fresh weight, 4.5 kg.) were used for the preparation of protein by a modification of the 'used ether-water' method of Chibnall, Miller, Hall & Westall (1933). Batches (500 g.) of seedlings were treated serially, cytolysed for 15 min. in 1 l. ether water and then pressed in a screw-plunger press, the expressed juices being returned to the ether water which was used for the next batch. The residue from the press was washed in 500 ml. tap water, pressed again, minced finely in a domestic mincer and extracted twice with 0.025M-Na₂B₄O₇ at pH 9.2 (Lugg, 1939), 500 ml. each time, the suspension being passed through a 1 mm. mesh wire sieve. The suspension was centrifuged at 400 g for 5 min. and then filtered through a pad of rammed paper pulp, 3 cm. thick. The combined filtrates were adjusted to pH 4.4 by the addition of about 190 ml. 2N-HCl. The protein was coagulated by heating the solution on a water bath to 80° for 5 min. and allowed to settle overnight in a large vessel. Most of the clear supernatant was then decanted and the protein was separated by centrifuging at 1500 g for 10 min. The preparation was extracted three times at the centrifuge with 0.005N-HCl, as recommended by Lugg (1938), and twice with 300 ml. absolute ethanol, held at 60° on a water bath for 30 min. After two washes with dry ether the preparation was dried at 80° in a ventilated oven and ground to a powder (5.09 g.).