CLXIV. CONSTITUENTS OF HUMAN SEMINAL PLASMA.

By MAURICE WALTER GOLDBLATT.

From the Sherrington School of Physiology, St Thomas's Hospital, London.

(*Received April 20th, 1935.*)

THE chemical description of human seminal fluid is far from complete, and except for isolated observations few quantitative data were available until recently. Landois and Stirling [1891] describe it as being neutral or alkaline and containing, in addition to 82 % water and 2 % salts (phosphates, sulphates, carbonates and chlorides), serum albumin, alkali albuminate, nuclein, lecithin, cholesterol and phosphorised fat. Slowtzoff [1916], cited by Roger, gives 90·3 % water, 0·9 % ash, 8·8 % organic matter, 2·58 % protein and 0·21 % lipoids. The ash is stated to contain K, Na, Ca, Mg, P, Fe and S; Ca and P being most abundant. The protein is described as albumin, globulin, nucleoprotein, nuclein and albumose. Cholesterol, choline and spermine are also stated to be present. McCarthy *et al.* [1928] made an extensive series of analyses of fluid obtained by massage of the seminal vesicles and prostate. It is certain that the fluid thus obtained contains a much greater proportion of prostatic secretion than the fluid normally ejaculated. Some of the analytical figures given by these authors are so widely different from those obtained with seminal fluid that this statement seems justified.

In a recent publication Huggins and Johnson [1933] have presented the results of analyses of various fluids obtained from the male genital tract: spermatocele fluid, semen, semen after vasotomy and seminal vesicle fluid. As we are here interested only in the seminal fluid, we shall refer to the results of these workers only in so far as they affect our knowledge of this fluid. They found that the volume and composition of semen from vasotomised men were not very different from those of semen from normal men, thus indicating that the contribution of fluid from the testes and epididymis is not considerable. They also found that whereas semen and seminal vesicle fluid are very rich in glucose and inorganic phosphorus, prostatic fluid contained no glucose at all and very little inorganic phosphorus. The fluid from the seminal vesicles possessed a composition not greatly different from that of semen itself, so that the authors conclude that the main contribution to semen is from the seminal vesicles. The average values for the constituents of semen calculated from the figures given by these workers are: p_H 7·26, non-protein N 66·3 mg./100 ml., CO_2 14·6 mM, Cl 42·6 mM, glucose 295 mg./100 ml., inorganic P 65·6 mg./100 ml., calcium 20·8 mg./100 ml. There are certain difficulties in making some of these analyses to which we shall refer later. It is of interest to compare these findings with the statement made by Marshall [1922] that the amount of calcium in one ejaculation is 0·01 g., *i.e.* the amount of calcium in 50 ml. semen according to the results of Huggins and Johnson. Unless the seminal fluid contains forms of calcium which cannot be precipitated by ammonium oxalate, the figure given by Marshall is 10 times too high.

The question of the nature of the proteins in seminal fluid has presented certain difficulties because of the presence of non-coagulable protein.

It has been stated by several workers that choline is present in seminal fluid. Evidence against this statement is given in a paper on the pharmacological properties of seminal plasma by the writer [Goldblatt, 1935].

Experimental.

The specimens placed at our disposal were the results of normal coitus and were received in the laboratory at various times up to 12 hours after ejaculation. In all the specimens motile spermatozoa were very abundant and in no case was there evidence of bacterial decomposition.

Having regard to the variable content of cells and other microscopically visible material, it was decided to work only with seminal plasma, *i.e.* the fluid obtained after centrifuging the semen for 10 min. or so at about 6000 r.p.m. and decanting or pipetting the supernatant fluid. Plasma thus obtained is almost free from cells and suspended particles. Seminal plasma is stable for long periods and appears rather like a milky blood serum; it is opalescent and often of a pale yellow colour; it is not very viscid and it gives no deposit on standing; sometimes it is very turbid; it possesses the peculiar odour of semen, which becomes more intense on warming.

Reaction. The p_H of seminal plasma, determined colorimetrically, varied in different specimens from 7·5 to 7·8, but this range is certainly more alkaline than at the time of ejaculation. Huggins and Johnson [1933] used semen immediately after emission and at once covered with oil and found the p_H to be 7·26, great care having been taken to prevent contamination with urinary residues. Such a low p_H compared with blood is in accord with the high amino-acid, phosphate and lactic acid contents.

It is generally stated that in physiological conditions the alkalinity of the seminal fluid is necessary to prevent immobilisation of the sperm cells by the acid secretion of the vagina. It must be remembered that the vagina itself is free from glands and that it is supplied with mucus from the glands of the cervix. The production of acid in the vagina is due to the action of organisms on the glycogen of the vaginal epithelium. It seems probable also that the spermatozoa on entering the vagina produce a considerable amount of lactic acid between the period of ejaculation and complete loss of motility. The necessity for a powerful buffering mechanism in semen is therefore obvious and even a rough titration with $N/10$ HCl shows how efficient its buffering capacity really is. Being, compared with blood, relatively poor in protein it owes its high buffering capacity to bicarbonate and to an enormous inorganic phosphate content. It seems that from the point of view of p_H regulation the seminal fluid stands intermediate between blood and urine, the mechanisms of both being utilised.

Bicarbonate. If seminal plasma be treated with a little mineral acid carbon dioxide is liberated. Experiments with the Van Slyke constant pressure apparatus show that seminal plasma loses half its bound CO_2 on exposure to a vacuum, from which we may conclude that the bound CO_2 is all present as HCO_3^-, as was to be expected. The average value obtained for our series of specimens was 22 millimols CO_2 per litre; this value is rather higher than that given by Huggins and Johnson, *viz.* 14·6.

Chloride. Chloride was estimated by Van Slyke's method for blood. The values obtained with three specimens were 0·209, 0·187 and 0·193 g./100 ml.,

expressed as NaCl, thus giving an average of 0·197 g./100 ml. The closeness of these results suggests that the salt content of seminal plasma may be normally maintained between as narrow limits as is the blood plasma chloride.

For prostato-vesicular secretion McCarthy *et al.* [1928] find 0·231 g./100 ml. for the chloride as NaCl whilst Huggins and Johnson [1933] give 0·164 for seminal vesicle fluid, 0·164 for semen after vasotomy and 0·355 for prostatic fluid. If we apply the figures of the latter workers to those of McCarthy *et al.* we find that the prostato-vesicular fluid obtained by the latter consisted of two parts vesicle fluid and one part prostatic fluid. The figures of Huggins and Johnson lead to the conclusion that, on the average, semen after vasotomy did not contain any prostatic fluid at all. An interesting possibility is thus presented that vasotomy may lead to an inhibition of the release of prostatic secretion. This is also suggested by the average figures of these authors for glucose and inorganic phosphorus.

Phosphate. Seminal plasma is very rich in phosphorus compounds. Attempts to use the technique of Briggs for the estimation of inorganic phosphorus led to unexpected difficulties; these were soon traced to the fact that the trichloroacetic acid filtrates were not protein-free. The same difficulty in obtaining protein-free filtrates is met with in each of the following methods of deproteinisation: Folin's tungstic acid, acidified sodium sulphate and colloidal ferric hydroxide, sodium hydroxide and zinc sulphate, 80 % alcohol. The soluble protein is proteose. In addition to this difficulty there is also the matter of the presence of spermine phosphate which is readily soluble in trichloroacetic acid. We shall consider the latter question first.

Behaviour of spermine phosphate towards Briggs's reagents.

In the first experiments we used spermine phosphate (kindly supplied by Messrs Hofmann La Roche) dissolved in dilute alkali. On adding the reagents in the proportions used for blood plasma a yellow crystalline precipitate was formed and a blue colour gradually developed in the turbid mixture. Removal of the precipitate and comparison of the blue colour with standards of KH_2PO_4 showed that from 1·35 and 0·68 mg. spermine phosphate (*i.e.* 0·16 mg. and 0·08 mg. P) only 0·07 and 0·04 mg. "inorganic" P was estimated. It was quite clear that the Briggs technique precipitated a great deal of the spermine as a molybdate insoluble in the conditions of the estimation.

Now the structure of spermine phosphate leads us to expect it to behave like an inorganic phosphate, so the following experiments were carried out to determine the proper conditions, using quantities of spermine of the same order as that found in seminal plasma. For these experiments we used a specimen of spermine diphosphate kindly supplied by Dr G. A. Harrison of St Bartholomew's Hospital and prepared by him from human tissues [1931].

A solution of this salt in 4 % trichloroacetic acid was prepared containing 1 mg. per ml. The formula for spermine diphosphate is $C_{10}H_{26}N_4 . 2H_3PO_4, 6H_2O$, *i.e.* it contains 21·3 % water of crystallisation and 12·25 % total P. Hence 1 mg. spermine diphosphate should yield 0·123 mg. "inorganic" P if it behaves as an inorganic phosphate.

(*a*) 2 ml. of the solution were wet-ashed and the total phosphorus determined by Briggs's method was found to be 0·256 mg. (theoretical 0·245).

(*b*) To determine the proportions of acid molybdate which, for quantities of spermine phosphate likely to be met with in human seminal plasma, would not produce either a turbidity or a precipitate, the following mixtures were prepared.

Spermine phosphate solution ml.	4% trichloro-acetic acid ml.	Briggs's acid molybdate ml.	Water ml.	Remarks
2·0	—	2·0	3·0	Precipitate
2·0	3·0	2·0	—	Precipitate
2·0	—	2·0	—	Turbidity
0·5	1·5	2·0	—	Turbidity
0·25	1·75	2·0	—	Turbidity
1·0	—	2·0	—	No turbidity
2·0	—	4·0	—	No turbidity

It is clear, therefore, that the use of Briggs's molybdate in trichloroacetic acid filtrates of human seminal plasma containing spermine will produce precipitates if the 2 : 7 proportion of molybdate be used as it is for blood plasma filtrates. If, however, two parts of molybdate be added to one part of the spermine-containing filtrate no precipitate or turbidity due to the spermine will be produced.

On mixing 1 ml. of the above spermine diphosphate solution with 2 ml. molybdate solution and then adding 1 ml. of the quinol (0·5 %) and 1 ml. of the sodium sulphite (20 %), a pure clear blue gradually developed which corresponded to an inorganic phosphorus content of 0·125 mg. (theoretical 0·123 mg.). Similarly 2 ml. of the solution of spermine diphosphate and 4 ml. of the molybdate followed by 1 ml. of the quinol and sulphite gave a blue corresponding to 0·227 mg. inorganic phosphorus (theoretical 0·245 mg.). The development of the colour ran exactly parallel with that of the standard KH_2PO_4, so that it is justifiable to suppose that spermine phosphate acts towards the Briggs reagents as an inorganic phosphate. If we take 3 mg. per ml. as the upper limit of the spermine phosphate content of seminal plasma, the above proportions of molybdate should obviate any difficulties from the formation of insoluble spermine phosphomolybdate, provided we take volumes of trichloroacetic acid filtrates equivalent to 2/3 ml. of seminal plasma. But when dealing with seminal plasma complications still arise from the presence of protein in the trichloroacetic acid filtrate. Huggins and Johnson [1933] refer shortly to a turbidity on adding acid molybdate to the trichloroacetic acid filtrate and state that they removed it by ultrafiltration through a collodion sac under a negative pressure of 150 mm. Hg. We must therefore consider what effect the presence of soluble protein (proteose) has upon the estimation of inorganic phosphorus.

Effect of proteose on the estimation of inorganic phosphorus.

A series of mixtures of 1 % Witte's peptone (filtered and shown to contain proteose and peptone) and KH_2PO_4 were prepared so that 2 ml. contained 0·1 mg. P and 0, 2, 4, 6, 8 and 10 mg. of the protein. To 2 ml. of these mixtures were added 10 ml. 20 % trichloroacetic acid. The protein precipitates were filtered and refiltered until the filtrate was as clear as possible. To 5 ml. of the filtrates were added 2 ml. acid molybdate, 1 ml. of quinol and 1 ml. of sulphite. In all except the first tube turbidities of increasing intensity appeared according to the amount of protein in the original tubes. After about 30 mins. the contents of each tube were filtered and the clear blue filtrates compared with the blue of the first filtrate. The following figures give the amounts of inorganic phosphate in the filtrates: 0·042 (no protein), 0·040, 0·035, 0·035, 0·030, 0·028 mg. It is clear that the losses were due to the formation of insoluble phosphomolybdate of protein. If acid molybdate be added to a solution of Witte's peptone a

precipitate is formed which readily dissolves in excess of the reagent; but if a trace of phosphorus be added to the protein solution before addition of the molybdate there is formed a precipitate which does not dissolve in excess of the reagent. In the 5 ml. portions of the trichloroacetic acid filtrates used in the above experiment there were increasing amounts of dissolved protein and so we expect increasing amounts of the phosphate to be lost. If the losses in inorganic phosphorus be plotted against the original concentration of protein the points fall approximately on a straight line. It is clear, therefore, that unless we know the amount of proteose in the filtrates obtained with seminal plasma an undetermined amount of inorganic phosphate will be lost in removing the precipitate formed on adding the acid molybdate. We have no data on the amount of proteose in seminal fluid but we suspect it to be present in very considerable amount. There is no doubt that the values obtained by other workers and ourselves for inorganic phosphorus in seminal fluid are considerably lower than the actual content. We proceed as follows: 10 ml. 20 % trichloroacetic acid are added to 2 ml. seminal plasma and after vigorous shaking allowed to stand for an hour. The mixture is now filtered and refiltered until a clear filtrate is obtained. This filtrate invariably gives a positive biuret reaction. To 2 ml. of the filtrate are added 4 ml. of the acid molybdate; a heavy yellowish turbidity forms at once and gradually settles out as an amorphous precipitate. Repeated filtration will usually yield a protein-free filtrate but sometimes we find it necessary to add a little $CaCl_2$ (anhydrous) and re-filter. Addition of the Briggs reagent to an aliquot part of the filtrate now gives a clear blue which can be compared with standards in the usual way.

In this way we obtain values for "inorganic" phosphorus which include all the spermine phosphate, but, as we have pointed out, these values are too low. The averages of our series of specimens were:

Total acid-soluble phosphorus	95 mg./100 ml.
"Inorganic phosphorus"	70 mg./100 ml.

If we take Harrison's figures for the spermine phosphate content of human semen, *viz.* 112–268 mg./100 ml., then it is not excessive to attribute some 15 to 30 mg./100 ml. P of the "inorganic" phosphorus to this substance. This leaves 40–50 mg./100 ml. as the true inorganic phosphate subject to the reservations considered above.

Huggins and Johnson [1933] found that prostatic fluid contained only 3·4 mg./100 ml. inorganic phosphorus. Harrison [1933] has shown that the prostate is richer in spermine than any other organ in the body; the average figure given was 583 mg. spermine phosphate per 100 g. wet weight. It is not unlikely that most of the phosphate in prostatic secretion is in the form of spermine phosphate. Now we have seen above that some 15–30 mg. of the "inorganic" phosphate of seminal fluid are due to spermine phosphate and there is no doubt that the prostatic contribution to seminal fluid is small in proportion to that of the seminal vesicles; hence we must conclude that the vesicles contain (secrete) spermine phosphate. The facts that the testes and epididymis contribute very little fluid to seminal fluid, that the testes are relatively poor in spermine (only 60 mg. per 100 g. wet weight according to Harrison [1933]) and that spermatocele fluid is free from spermine lead us to the conclusion that the major part of the spermine in seminal fluid arises from the seminal vesicles. Fluid from the distended seminal vesicles of young men was obtained by Huggins and Johnson [1933] and found to contain 45 mg./100 ml. inorganic phosphorus, but they did not consider the spermine content.

There is no direct evidence as to the function of all this phosphorus but it seems reasonable to consider the great change in chemical environment experienced by the sperm on ejaculation as related to the necessity for active movement in the female genital tract and that the phosphates ensure a very efficient buffering action. As to the function of spermine phosphate nothing is known.

Calcium. The only other inorganic constituent of seminal plasma we examined was calcium. Using the ordinary technique of Kramer and Tisdall for serum calcium we found an average value of 24·5 mg./100 ml. This agrees well with the finding by Huggins and Johnson of 20·8 mg./100 ml. The origin of this very high concentration of calcium is most probably the prostate. This is supported by the finding of McCarthy *et al.* [1928] of 66 mg./100 ml. calcium in prostato-vesicular secretion. The former authors, however, give only 23·3 mg./100 ml. as the calcium content of seminal fluid after vasotomy, a result due probably to the small fluid contribution made by the prostate.

The forms in which the calcium exists in seminal fluid are probably calcium hydrogen phosphate, calcium bicarbonate and a combination with protein. The low phosphate content of prostatic secretion indicates that very little calcium can come from this source as the hydrogen phosphate but we have no certain data as to the partition of calcium among the three possible combinations.

Urea. It is not surprising to find urea in seminal fluid having regard to its ready diffusibility into all tissues but its function here, if any, is not easily understood. The great concentration of other blood constituents in the seminal plasma may involve urea more or less accidentally. Be that as it may, the concentration of urea in this fluid is roughly twice that in blood. The average value in our series was 72 mg./100 ml., estimated by hydrolysis with soya bean and aeration into standard acid. Of course very small contaminations with urine would give false values but the evidence is against such contamination since similar values are obtained even when the urethra was washed with dilute boric acid before ejaculation [McCarthy *et al.*, 1928].

Reducing substances. In estimating the reducing power of seminal plasma the difficulty of deproteinising, already referred to, at once introduces complications. We have used MacLean's method (a Cu method) and that of Hagedorn and Jensen (ferricyanide method). The latter method gives values which are certainly unreliable owing to the much greater proportion of protein which escapes precipitation. The former method gives filtrates which contain only a faint trace of protein and, provided a "blank" determination be made in the cold, reliable values are obtainable. The following values were obtained with the seminal plasma of normal and diabetic subjects.

Normal, 206, 277, 226, 211, 282, 380, 291 mg./100 ml. as glucose.
Diabetic, 628, 569, 576, 669, 681 mg./100 ml. as glucose.

Thus normal seminal plasma contains between two and three times the amount of reducing substances found in normal blood. The values for the diabetic subjects are probably more or less misleading, since traces of glucose are always to be found in the urethra of the glycosuric. There can be no reasonable doubt that the sugar in the seminal fluid must be regarded as a nutrient for the spermatozoa and it is easy to demonstrate the utilisation of the sugar if the sperm cells are kept alive. If the sperm cells are killed by heat, dilution or by the addition of a drop of toluene no fall occurs in the reducing power of the fluid. Nor does the reducing power change in the seminal plasma if it is kept sterile. In the following experiment a fresh specimen of human seminal fluid was obtained in a sterile tube, divided into three parts: one part was heated to kill the sperm,

another part incubated in a water-bath at 38° and the remaining part centrifuged and the supernatant plasma used. Estimations of reducing substances were made in each part at once and after 20 hours' incubation.

	Heated fluid	Plasma	Unheated fluid
At once	291	280	291 mg./100 ml. as glucose
After 20 hours' incubation	289	280	72

McCarthy *et al.* [1928] also recorded glycolytic activity in incubated seminal fluid and found that there was a large increase in lactic acid but that this increase did not account for the loss in sugar. This production of lactic acid is probably an important factor in the poor survival of spermatozoa in natural seminal fluid *in vitro* however carefully sterility, temperature and oxygen supply are controlled. Failure of the buffering capacity comes about sooner or later but generally within 12 hours.

The source of the sugar in the seminal fluid seems to be the seminal vesicles, for the prostatic fluid has no reducing power at all.

Lactic acid. Seminal fluid contains lactic acid in large amounts. The acid is present in the freshly ejaculated fluid and increases on incubation. We used the method of Friedemann, Cotonio and Shaffer for the determination of this substance in the tungstic acid filtrate after treatment with copper sulphate and calcium hydroxide. The following values were obtained with different specimens of fresh seminal fluid, the spermatozoa having been killed soon after ejaculation:

92, 80, 104, 87, 103 mg./100 ml.

The increase in lactic acid during incubation of seminal fluid containing living sperm cells is shown in the following:

	Reducing power as glucose mg./100 ml.	Lactic acid mg./100 ml.
At beginning	291	80
After 20 hours' incubation at 38°	72	140

There is clearly a great discrepancy between the lactic acid production and the lost glucose. How the lactic acid is produced in the vesicles it is hard to say. The few spermatozoa which may be present in this situation do not appear to be sufficient to account for so high a lactic acid value nor can we attribute it to an enzymic action on the glucose, for the seminal plasma, freed from spermatozoa, does not show any increased lactic acid content after incubation. Nor can we suppose that it is formed in the prostate (because prostatic secretion is free from reducing substances) unless we consider that it may arise from a non-carbohydrate source (*e.g.* amino-acid). If it derives directly from the blood we must attribute remarkable powers of selective concentration to the seminal vesicles. Teleologically it appears useless to produce large quantities of lactic acid in a medium where its presence later will be inimical to the survival of the sperm cells. There are, of course, considerable numbers of leucocytes and other cells in seminal fluid and these, as in blood, may be partly responsible for the glucose-lactic acid reaction. The matter is not easy to investigate and is in a state of obscurity.

Cholesterol. This substance can be readily estimated in seminal plasma by the usual methods for serum cholesterol. Sometimes other substances are extracted by the chloroform and may interfere with the purity of the colour obtained with the acetic anhydride. The average value for our series was 82 mg./100 ml.

In the following table we give a comparison of the composition of blood plasma and seminal plasma.

Composition of blood and seminal plasma.

	Blood plasma 100 ml.	Seminal plasma 100 ml.
p_H	7·4	7·2–7·3
NaCl (g.)	0·59–0·60	0·20
CO_2 as ml./100 ml.	50– 70	50
Inorganic P (mg.)	3– 4	40– 50
Acid-soluble P (mg.)	3– 4	95
Spermine P (mg.)	—	15– 30
Calcium (mg.)	10	24– 25
Glucose (mg.)	100	200–300
Urea (mg.)	30	72
Lactic acid (mg.)	15– 20	90–100
Cholesterol (mg.)	150–200	80

Proteins. Seminal plasma gives all the protein colour reactions. When slightly acidified with 1 % acetic acid it gives a slight precipitate; this probably indicates the presence of a small amount of nucleoprotein. It must be remembered that the mucinous matter secreted by the accessory glands is removed in a stringy mass when the semen is centrifuged. On dilution of seminal plasma with a large excess of water a slight precipitate of globulin is formed. On filtering off the globulin a considerable amount of coagulable protein is readily demonstrable in the filtrate; this we take to be albumin.

The reaction at which maximum coagulation is obtained is about p_H 5·5 but the amount of coagulable protein seems to vary a great deal in different specimens. Removal of the coagulable protein always yields a richly proteinous filtrate. This non-coagulable protein is referred to in the older literature as propeptone and sometimes as hemialbumose. Marshall [1922] refers to an albumose-like substance and remarks that "the statement that this protein is an albumose is probably not correct, since albumoses have never been found to occur in the living cell". Posner [1892] refers to the non-coagulable protein of human seminal fluid as propeptone and finds that it is absent from spermatocele fluid which contains enormous numbers of fully developed but non-motile spermatozoa; he concludes that the propeptone is elaborated in the accessory glands and not in the testes. Vertun [1899] also analysed spermatocele fluid; in his case actively motile spermatozoa were present; the fluid contained albumin and a trace of globulin but a variety of tests showed that it was free from albumose, from which he concluded that the presence of albumose was of no importance in the maintenance of the motility of the spermatozoa.

Our own findings with every specimen of seminal plasma support the view that there is proteose present. On removing the coagulable protein by boiling at p_H 5·5 and filtering, the filtrate gives a pink biuret reaction; half-saturation of the filtrate with ammonium sulphate gives a considerable precipitate the filtrate from which gives another precipitate on full saturation with ammonium sulphate. The filtrate after full saturation gives no protein reactions. We were able partly to separate the primary and secondary proteoses by ultrafiltration. Human seminal plasma was ultrafiltered through a collodion membrane under a pressure of 50 mm. Hg. A yellowish clear filtrate was obtained which was alkaline to litmus, contained no coagulable protein, gave a pink biuret reaction, a positive salicylsulphonic acid reaction, a precipitate with nitric acid which disappeared on warming and reappeared on cooling but no precipitate with copper

sulphate. The ultrafiltrate on half-saturation with ammonium sulphate gave a copious precipitate the filtrate from which still contained protein; all the protein was removed on full saturation of the filtrate. The ultrafiltrate, therefore, contained both primary and secondary proteoses. The fluid left in the collodion sac was acidified and boiled to remove the coagulable proteins; the filtrate from this was rich in protein but gave no precipitate on half-saturation with ammonium sulphate but a copious one on full saturation. It seems, therefore, that the primary proteose had all passed through the collodion filter but the secondary proteose had done so only in part. Some specimens of seminal fluid contain no primary proteose at all but there is never any doubt about the secondary proteose. At least two secondary proteoses are present which can be demonstrated as follows. Remove the coagulable proteins by boiling at p_H 5·5; filter and half-saturate the filtrate with ammonium sulphate; filter and add a half-volume of saturated ammonium sulphate; a copious precipitate is produced. This precipitate can readily be filtered off, dissolved in water and precipitated with two volumes of absolute alcohol (the alcohol precipitates the ammonium sulphate as well as the protein but a pink biuret reaction is readily obtained if excess of alkali is used). These facts show the presence of thioalbumose or a substance closely resembling it. On removing the thioalbumose we obtain a water-clear filtrate which gives a slight turbidity or precipitate on full saturation with ammonium sulphate, removal of which yields a non-protein filtrate. The protein nature of the last precipitate is readily established by dissolving in water and obtaining a pink biuret reaction. We did not further identify this substance but it seems likely that it is synalbumose.

We conclude that the following proteins are present in seminal fluid: mucin, nucleoprotein, albumin, globulin (traces), proteoses but no peptone. The proteoses may be both primary and secondary but in some specimens primary proteose may be absent. Secondary proteoses are always present and consist of thioalbumose and synalbumose or bodies closely resembling them.

It is now clear why the usual methods of protein precipitation used in blood analysis do not yield non-protein filtrates when applied to seminal plasma. These methods if applied to a solution of Witte's peptone, containing proteose and peptone, always yield filtrates giving the reactions of proteose. The same remarks apply if the precipitation is carried out in 80 % alcohol.

Alcohol-soluble material in seminal plasma.

One volume of seminal plasma is mixed with four volumes of absolute alcohol and well shaken. The copious white precipitate is removed by filtration and the alcohol removed from the filtrate by distillation at 42° under reduced pressure. The whitish residue when taken up in water yields a turbid suspension and gives the following reactions: it is alkaline (sometimes neutral), biuret—pink, Millon—weakly positive, xanthoproteic—no precipitate but turns yellow on heating and orange on adding ammonia, Molisch and glyoxylic—negative, ammonia does not give a precipitate, the Pauly, Sakaguchi and ninhydrin reactions are all strongly positive. The intensities of the last three reactions are so out of proportion to that of the biuret reaction that it seems possible that there are free iminazole and guanidine compounds present. This possibility has not as yet been followed up but it is suggestive that all these reactions become negative in the extract after a relatively short period of dialysis and that after precipitation of all the proteins of seminal plasma by full saturation with ammonium sulphate the filtrate still gives these reactions. We have already

referred to the results of other workers that seminal fluid is very rich in amino-acids and this may explain our findings. We conclude, therefore, that the alcohol extract contains proteose and probably a considerable amount of amino-acids.

The presence of proteose in seminal plasma suggests that a proteolytic enzyme may be present or that the spermatozoa may have the power of splitting proteins. The latter seems improbable in view of the absence of proteose from spermatocele fluid containing motile sperm. The absence of peptone argues against any ordinary peptic activity but the matter requires further investigation. Tested upon calcified milk seminal plasma gives no evidence of the presence of a proteolytic ferment.

Diastase. Karassik [1927] reports the presence of diastase in human prostatic fluid and McCarthy *et al.* [1928] found it in prostato-vesicular fluid. The wide distribution of this enzyme renders its presence in seminal plasma not unexpected. We studied the diastatic power of fresh seminal plasma in buffered solution as follows:

Tube no.	1	2	3
0·1 % starch (ml.)	5	5	5
1·0 % NaCl (ml.)	2	2	2
KH_2PO_4-NaOH buffer (ml.)	2	2	2
p_H	6·6	5·8	8·0
Seminal plasma (ml.)	0·4	0·4	0·4
Time for complete digestion at 38° (min.)	45	65	No change in 2 hours

It appears, therefore, that a diastase is present which is active at reactions between p_H 6 and 7. A simple calculation shows that the diastatic activity of 3 or 4 ml. of seminal plasma is sufficient to produce an amount of simpler sugars from polysaccharide (*e.g.* glycogen from the vagina and cervix) more than sufficient to supply the needs of the spermatozoa for a considerable time.

Glycolytic activity. Seminal plasma has no glycolytic activity if all the sperm cells have been removed.

Thrombokinase. Coagulating agents have been stated to be present in the seminal secretions of rats, mice and guinea-pigs by Walker [1910] and others. A special gland exists in these animals which secretes a substance which coagulates the contents of the seminal vesicles and so brings about the vaginal plug formation well known in these animals. Walker could not find these glands in rabbits or bullocks. In human seminal fluid particles or aggregations are seen which suggest some sort of coagulation to this author but he does not state what is coagulated. Any close analogy to the fibrinogen-fibrin change in blood seems improbable but, although the amount of globulin in seminal plasma is small, we cannot say with certainty that something akin to fibrinogen is not present. One factor in the mechanism of blood coagulation can readily be demonstrated in seminal plasma, *viz.* thrombokinase. This was done as follows: to 4·5 ml. seminal plasma were added 27 ml. absolute alcohol; the mixture was well shaken and allowed to stand for an hour or so; the filtrate from this was freed from alcohol by distillation at 42° under reduced pressure and the residue taken up into 10 ml. water. A solution of prothrombase (Mellanby) containing 0·5 mg./ml. was prepared. The presence of thrombokinase was tested by the power to activate prothrombase to thrombase in the presence of $CaCl_2$; the thrombase activity was tested by the power to coagulate oxalated ox plasma. The prothrombase was first shown to be inactive. A mixture of 2 ml. prothrombase

solution, 1 drop $N/5$ $CaCl_2$ and 1 drop of seminal plasma extract was placed in a water-bath at 38° for 2 mins. 0·1 ml. of the prothrombase solution thus treated added to 1 ml. oxalated plasma at 38° produced complete coagulation in 15 secs. An exactly similar experiment with a freshly prepared aqueous extract of bull's testis gave a coagulation time of 12 secs. An acetone extract of seminal plasma was also used in a similar experiment and gave a coagulation time of 45 secs.

It is thus clear that a thrombokinase of considerable activity is present in seminal plasma. The origin of this kinase may be the testis but we think its more likely origin is the broken down cells of several kinds which are always found in the seminal fluid. As to the function of kinase in seminal fluid we make no suggestion.

Miscellaneous observations.

(*a*) It seemed possible that seminal fluid might contain hormones having an influence on the female genital tract. Injection of 1 ml. fresh seminal fluid intravenously into young virgin rabbits did not, however, produce any changes in the uterus or ovaries during periods up to 48 hours after the injections. The animals showed no toxic effects but invariably the injections were followed by an almost immediate defaecation; for the effect on intestinal muscle see Goldblatt [1935].

(*b*) Successive intravenous injections of seminal fluid or alcoholic extracts thereof into rabbits had no effect on blood sugar.

(*c*) Seminal fluid does not give the nitroprusside reaction for glutathione or related bodies.

Summary.

1. Human seminal plasma has been quantitatively analysed for certain constituents. The ratios of the concentrations of these in seminal plasma to their concentrations in blood plasma are as follows: chloride and cholesterol, 0·3–0·5; bicarbonate, 0·7–1·0; calcium, glucose, urea, 2·0–3·0; lactic acid, 5·0–6·0; acid-soluble phosphorus (inorganic, 50 %, spermine, 30 %, undetermined 20 %), 30.

2. The proteins of seminal plasma include: mucin (traces), nucleoprotein, albumin, globulin (traces), proteoses. The proteoses may be both primary and secondary; secondary proteose is always present. The secondary proteose, it is suggested, consists of thioalbumose and synalbumose or bodies closely resembling them. Peptone is never present in seminal plasma. The presence of proteoses interferes with several estimations.

Protamines and histones have not been found in seminal plasma.

3. Glycolysis can be demonstrated in seminal plasma only if active sperm cells are present. This is associated with a large increase in lactic acid but the latter is not sufficient to account for all the lost reducing power.

Proteolytic ferments have not been found in seminal plasma and hence the presence of proteose cannot be explained on enzymic grounds.

Diastase and thrombokinase have been demonstrated in seminal plasma.

4. Seminal plasma does not appear to contain hormones affecting either the genital tract or carbohydrate metabolism.

REFERENCES.

Goldblatt (1935). *J. Physiol.* **84**, 208.
Harrison (1931). *Biochem. J.* **25**, 1885.
—— (1933). *Biochem. J.* **27**, 1152.
Huggins and Johnson (1933). *Amer. J. Physiol.* **103**, 574.
Karassik (1927). *Z. ges. exp. Med.* **53**, 734.
Landois and Stirling (1891). Text-book of physiology. (C. Griffin and Co., London.)
Marshall (1922). The physiology of reproduction. (Longmans, Green and Co., London.)
McCarthy, Stepita, Johnston and Killian (1928). *J. Urology*, **19**, 43.
Posner (1892). *Zentr. mikr. Wiss.* **13**, 225.
Slowtzoff (1916). Cited by G. H. Roger, Traité de physiologie. (Masson et Cie, Paris.)
Vertun (1899). *Zentr. mikr. Wiss.* **31**, 529.
Walker (1910). *Bull. Johns Hopkins Hosp.* **21**, 182.