THE ISOLATION OF SPERM ANTAGGLUTIN FROM THE FOLLICLE FLUID, AND SOME OF ITS PROPERTIES*

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In the sperm plasm and on the surface of the heads of the spermatozoa of most mammals a factor occurs that has the ability to prevent and abolish the well-known head-to-head agglutination[1,2]. When this factor, which has been termed "sperm antagglutin", is absent or present in its oxidized, biologically inactive form, the spermatozoa agglutinate in a special maturation state. Male sperm antagglutin is formed in the prostatic gland in cattle[3] and man[3]. It is a proteid, containing an active group of which carbohydrate residues, sulphuric acid residues, and a carbocyclic derivative, showing similarities to the tocopherols, are constituents[2,4,5]. Most striking physico-chemical properties are its surface activity and metachromasia when in the reduced form, and the fact that it is salted-out by rather low salt concentrations (1.3% NH_4OH, pH 7.8)[2].

A sperm antiagglutinic factor was observed also in the female genital tract, for the first time in the tubal secretion from the cow in oestrus[1]. Later it was demonstrated that it is formed in the follicles of man, cattle, swine, rabbit, and rat, and in the Fallopian tubes in the rabbit[6] and in man[7].

Like male antagglutin, the female substance is not species-specific in its biological action. This makes it possible to use bull spermatozoa in a biological test for female antagglutin from other species. Such a method was worked out for male antagglutin by LINDAHL AND KIHLSTRÖM[2]. An antiagglutinic index (AAI) is calculated as the quotient of the difference between the percentage of agglutinated spermatozoa before and after the action of the antagglutin in the sample, and the percentage of agglutinated spermatozoa before this action. This test, which under favourable conditions gives AAI-values roughly proportional to the concentration of male antagglutin, has been used throughout in the isolation of the female antagglutin as the guiding reaction. An addition of a surplus of thioglycolate secures reduction of the antagglutin when this test is performed. However, as will be seen later, the proportionality between index and concentration is limited for the female antagglutin to a very narrow range, which fact of course greatly reduces the possibility of estimating quantities of antagglutin by this method. A difference in index of more than 0.10 may be considered as significant. In the present investigation we furthermore used as a chemical qualitative test the reaction that forms the base of the analytical method of LINDAHL, KIHLSTRÖM, AND ROSS[8] for male antagglutin (*cf.* below).

As a medium for all our experiments with living spermatozoa reported in this

* Although this work is not definitive in some parts it has to be published now as one of the authors (A. N.) is taking up a new position in another laboratory.

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paper a modification of the diluter of PHILLIPS AND SPITZER[9] was used, composed of glucose, galactose, and phosphate buffer, in the proportions given.

THE ISOLATION

To begin with, we tried to isolate the antagglutin from cows' oestrus mucus, the antiagglutinic effect of which is very high. However, this material varied much from sample to sample, offering very changing conditions. As the fluid from growing follicles of the cow appeared to give a rather strong antiagglutinic effect, we later changed to this material, which was obtained by puncturing the follicles and withdrawing the liquid with an injection syringe. The fluid from large follicles occasionally agglutinates spermatozoa; this always happens with the fluid from follicular cysts. In such cases agglutinating substances prevail over the antagglutin, but the latter can still be isolated.

According to electrophoretic experiments on filter-paper performed for 60 to 70 hours at different pH (3.3–8.6) the antagglutinic substance of the follicle liquid did not appear to be associated with any protein component. The antagglutinic activity was transported simultaneously towards both the anode and the cathode, chiefly independently of the pH and the current density used, whereas the proteins travelled in distinct bands in close dependence on the experimental conditions mentioned. These observations were further supplemented by experiments in which the antagglutinic substance was dialyzed through a cellophane membrane. Nine ml follicle liquid was dialyzed against 30 ml Ringer solution, pH 7.1, for 20 hours at $+3^{\circ}$ C. The dialysate was subjected to the biological antagglutin test, which gave an AAI of 0.43. The qualitative chemical test was positive. By ultra-filtration of follicle liquid through a collodion membrane* (Membranfiltergesellschaft, Sartoriuswerke, Göttingen) a filtrate was obtained that also gives a positive result with the qualitative chemical test. All these observations indicate that a possible combination of the antagglutinic principle with any protein of the follicle liquid is a very loose one, both at physiological pH and in normal salt medium.

Unlike the male antagglutin the female antagglutin is not salted-out by low salt concentrations. Not until a concentration of 15–25% at pH 3.0–4.5, and of 30–40% at pH 7.8, is reached do protein-containing fractions with antiagglutinic activity appear. Probably part of the antagglutin is adsorbed to the protein precipitate salted-out within these broad concentration ranges. Isolation by salting-out was therefore abolished.

As the solubility of the female antagglutin in different solvents gradually became known an attempt was made to isolate the substance with the aid of fractionated extraction. By addition of 9 parts of ethanol to 1 part of follicle fluid all proteins were precipitated and then spun down. No antagglutin was adsorbed on this precipitate. The yellow supernatant, containing antagglutin, lipids, sugar, peptides, amino acids, etc., was evaporated to dryness. By extraction with anhydrous ethanol, ethyl ether, or carbon tetrachloride, the lipids and the coloured substance, which appeared to be a carotene, were removed without too heavy losses of antagglutin. The remaining residue was then easily dissolved in 90% ethanol, the solution having an absorption

* The maximum size of particles passing this membrane corresponds to a molecular weight of 4,000.

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maximum at 290 and a minimum at 260 mμ. However, since it appeared later that this treatment changed the rate of dissolution in water of the antagglutin (*cf.* below) further purification along these lines was abandoned.

Adsorption on different adsorbents, *e.g.* calcium carbonate, celite, quartz, was tried. Quartz appeared to be most advantageous, and the following procedure was worked out: the pH of the follicle fluid is adjusted to 8.8 with NaOH, and 50 ml of the liquid run through a 20 cm × 2.5 cm column of coarse quartz powder. The column is then washed with 200 ml NaOH-solution, pH 8.6, and eluted with carbon dioxide containing redistilled water (pH 5.5). The first part of the eluate, being alkaline, is rejected and stored for repeated adsorption. When the pH of the eluate has dropped to about 5.5, fractions of 100 ml are taken. The first generally contains substances with an absorption maximum at 275–280 mμ and a minimum at 250 mμ. These substances, which are not associated with the antagglutinic effect, are ninhydrin-positive; they accompany the female antagglutin in other procedures of isolation also. When these contaminations have left the column the subsequent fractions seem to contain the antagglutin in a rather pure form as far as organic material is concerned.

The elution with water containing carbon dioxide is very slow, but has the advantage that no non-volatile substances are introduced. Addition of ethyl ether to the eluant very much accelerates the elution; for reasons to be explained later (*cf.* below), this ought to be avoided. The yield of the described isolation procedure is probably low. From 1,000 ml follicle fluid about 3 mg substance were obtained. As all our efforts were concentrated upon obtaining a substance homogeneous with respect to the organic material we took no particular steps to avoid inorganic impurities. Thus, partly as a result of using a distilled water of inferior quality for the elution and concentrating large quantities of eluate, our samples of the isolated substance had a high content of inorganic material. The described procedure is being developed further.

SOME CHEMICAL PROPERTIES OF FEMALE SPERM ANTAGGLUTIN

The female antagglutin isolated by the above method is very hygroscopic and soluble in distilled water. It is also to some extent soluble in apolar or slightly polar solvents, such as carbon tetrachloride, petrol ether, ethyl ether, and acetone, and somewhat more soluble in the more polar solvents methanol and ethanol. Once dissolved in these slightly polar or non-polar solvents, the antagglutin, after they have evaporated, forms upon the inside of the vessel an unwettable coating that dissolves only slowly in water. For methanol and ethanol this holds only when the alcohols have been used in an anhydrous form. Treatment of the antagglutin with ethanol-water mixtures, *e.g.* 90% ethanol, does not produce this effect. On the other hand, the change in rate of dissolution is induced by water saturated with ethyl ether. Admixture of this substance to the eluant in the isolation procedure described above should therefore be avoided, as should in general extractions with slightly polar or non-polar solvents as long as the nature of the mentioned change is not known. As a matter of fact, the female antagglutin is insoluble in pyridine.

The female antagglutin has a comparatively small molecule escaping as it does through membranes the pores of which allow the passage of particles up to a molecular weight of 4,000.

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The absorption curve in ultraviolet of female antagglutin (Fig. 1) agrees well in its main course with that of the male antagglutin. From 220 mμ it descends without maxima or minima with increasing wavelengths, but has an inflexion point at 260 mμ. Compared with the absorption curve of male antagglutin it is much lower at 220 mμ. This difference is in accordance with the absence of a protein residue in the female substance. On acount of the relatively high admixture of salts not too much weight should be attached to the form of the absorption curve.

The purified antagglutin was subjected to microanalysis for ash, nitrogen, phosphorus, and sulphur. Nitrogen was analysed according to KIRSTEN AND GRUNBAUM[10], sulphur according to KIRSTEN[11], and phosphorus according to WEIL-MALHERBE AND GREEN[12]. All analyses were performed by Dr. W. J. KIRSTEN, Uppsala. A sample of 0.504 mg gave 0.196 mg of ash (39%). According to flame spectrophotometric determinations, the chief metallic constituent of this ash was magnesium, but iron, nickel, chromium, and sodium, were also present. Possibly the preparations are hygroscopic owing to contamination with magnesium chloride. Sulphur was determined in the sample, and less than 1 μg, *i.e.* less than 0.2%, was found. From the same preparation a sample of 0.544 mg gave 0.01099 ml nitrogen (770 mm Hg), corresponding to 0.02%. The contamination with salts of a similar preparation was reduced by dissolving the substance in anhydrous ethanol and precipitating salts by addition of an equal amount of ethyl ether. Of this sample 0.177 mg was analysed for phosphorus. No trace could be detected, *i.e.* the sample contained less than 0.1% phosphorus.

Female antagglutin gives a positive Molisch reaction, indicating the presence of saccharides. With the benzidine reagent, which according to HORROCKS[13] is specific for reducing sugars, the antagglutin gives a positive reaction only after hydrolysis.

The solubility of the female antagglutin in apolar solvents indicates that it contains a lipophilic residue also. The following experiment suggests that this residue is related to the corresponding derivative in the male antagglutin. Follicle fluid was precipitated with ethanol as described above (p. 23), and the precipitate spun down. The supernatant was evaporated to dryness, and the solids extracted with water. This solution was mixed with an equal volume of 0.2 *M* lead acetate. A white precipitate was formed. This was spun down, silted up in distilled water, treated with hydrogen sulphide for three hours, and left overnight. The lead sulphide formed was

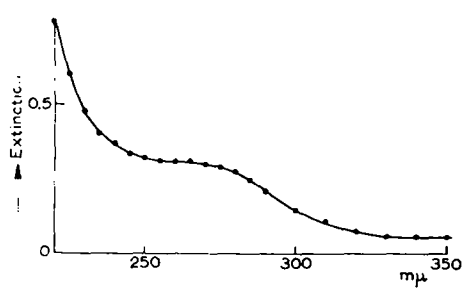

Fig. 1. Absorption curve in ultraviolet light of purified female antagglutin in distilled water. The preparation was contaminated with salts.

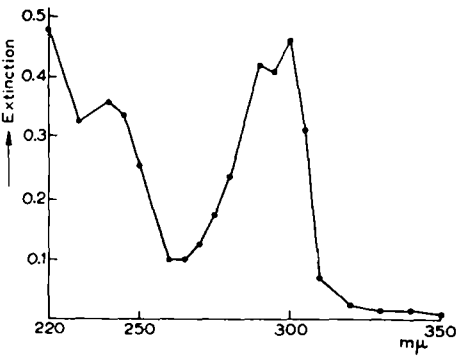

Fig. 2. Absorption curve of slightly denaturated female antagglutin in watery solution.

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removed by centrifugation, and the solution freed from hydrogen sulphide by aeration. This solution had an antagglutinic activity (AAI = 0.21); its absorption spectrum had maxima at 240, 290, and 300 mμ (Fig. 2) as has a slightly denatured form of the "active group" of the male antagglutin, described by LINDAHL, KIHLSTRÖM, AND ROSS[5*]. Unfortunately, this result could not be reproduced, although the experiment was repeated several times. Most probably, however, the substance having the absorption spectrum mentioned above originated from the antagglutin.

The procedure used as a quantitative method for the determination of male sperm antagglutin[8] has been applied to different kinds of samples containing female antagglutin. When the isolated substance was used the absorption curve (Fig. 3) was very similar to that obtained with male antagglutin. As with the male substance, the absorption peak disappears at further oxidation (*cf.* p. 27). Very small quantities were, however, used for such experiments. The quantitative relationships were in-

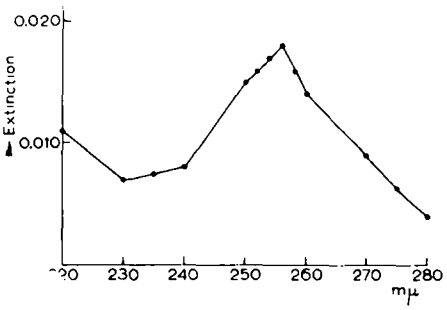

Fig. 3. Absorption curve of product obtained by applying the quantitative method for male antagglutin[4] to female antagglutin chromatographed on quartz.

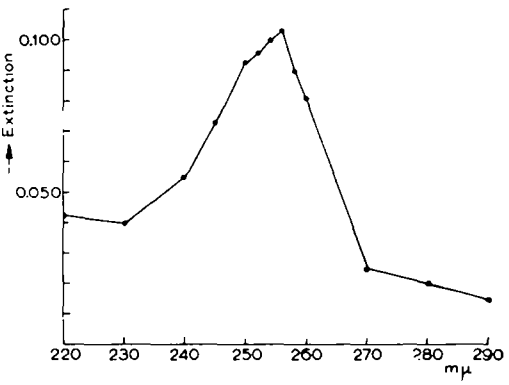

Fig. 4. Absorption curve of product obtained by applying the quantitative method for male antagglutin[4] to female antagglutin isolated by fractionated extraction with ethanol, ethyl ether, and water.

vestigated on two different preparations. The differences between extinction maximum and minimum were proportional to the quantities taken, as long as they originated from the same preparation. Evidently the two preparations differed in the concentration of antagglutin.

An absorption curve of this kind, *i.e.*, with a comparatively low background, obtained with a large quantity of antagglutin is shown in Fig. 4. This material had been purified in another way, involving chiefly precipitation of follicle fluid with 90% ethanol, and precipitation of impurities from the ethanolic solution by the addition of ethyl ether. After this solvent had been evaporated off, extraction with water dissolved only part of the antagglutin. The analytical procedure was applied to the remainder, which formed a fatty coating on the glass vessel (*cf.* p. 24). The asymmetry shown in this curve, and also in several others obtained with material prepared in a similar way, may be due to some impurity.

Since the cervical mucus shows in the antiagglutinic activity variations related

* Actually the first-mentioned maximum of this compound appears only as a bulge in the figure of the paper quoted. It had, however, been observed earlier[2].

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to the phases of the menstrual cycle[11], it seemed to be of interest to apply the mentioned procedure to specimens taken at or shortly after ovulation, showing high AAI-values, and during the later part of the cycle, with low or negative AAI-values.

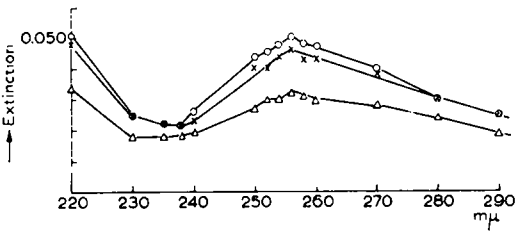

Fig. 5. Absorption curve of product obtained by applying the quantitative method for male antagglutin[4] to human cervical mucus from the 16th day of the cycle. The lower curve was obtained directly; after aeration for twenty minutes with colloidal platinum the same material gave the middle curve, and after another twenty minutes aeration the top curve. Further aeration reduced the maximum-minimum difference.

In the first group the differences very markedly deviated from zero; this was not the case in the second group. As can be seen from Fig. 5, the oxidation effected by the manganese dioxide added[8] was not sufficient, since aeration with platinum as catalyst further raised the extinction. In each case the rise was proportional to the original value, and was thus insignificant or nil for samples from the late part of the cycle.

ON THE BIOLOGICAL ACTIVITY OF THE FEMALE ANTAGGLUTIN

Like the male, the female antagglutin exerts its biological effect only in a reduced state. Within certain limits the inactivation by oxidation is reversible. The reduction may be performed with sulphhydryl-compounds, ascorbic acid or catalytically with hydrogen. The oxidation of the antagglutin by oxygen (air) is accelerated by the addition of a catalyst, *e.g.* colloidal platinum, and by raising the temperature of the solution to 70° C. Higher temperatures are deleterious to the biological activity*, as is repeated freezing and thawing. The reversibility of the oxidation is demonstrated in Fig. 6 by an experiment with isolated antagglutin, to the aqueous solution of which some colloidal platinum had been added. Treatment with oxygen alternating with hydrogen made the AAI decrease or rise, respectively. However, in the normal follicle fluid the antagglutin is protected against oxidation. Aeration for one hour of follicle fluid to which colloidal platinum had been added only lowered the AAI from 0.20 to 0.15, *i.e.* 25%. A similar experiment with human cervical secretion from the fourteenth day of the cycle is given in Fig. 6. Here 0.028 ml secretion was shaken vigorously for 15 minutes with 0.50 ml diluter. After centrifugation the antagglutinic index of the supernatant was determined, both at once, and after treatment with oxygen and colloidal platinum for various periods of time. As can be seen from Fig. 7, the biological activity is unchanged after half an hour, and has decreased only about 25% after another half hour (*cf.* corresponding experiment with follicle fluid).

As can be seen from Fig. 6, this preparation not only loses its antiagglutinic effect when being oxidized, but also acquires the property of being slightly agglutinating. This has been more pronounced in some experiments with other preparations (of lower purity?). In the follicle fluid substances occur which under certain conditions may acquire the property of being strongly agglutinating. Follicle fluid, having a marked antiagglutinic effect when freshly taken, often induces strong agglutination after 12 hours standing at 3° C and also when reduced afterwards by addition of

* Possibly by accelerating an irreversible oxidation.

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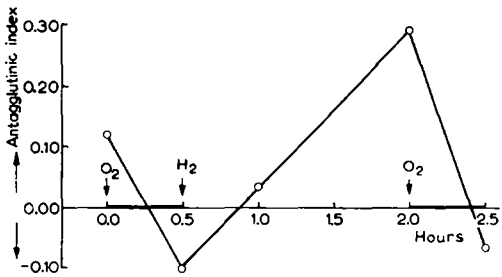

Fig. 6. Changes in biological activity of isolated antagglutin at oxidation and reduction. Oxygen and hydrogen respectively, was bubbled through a watery antagglutin solution at room temperature.

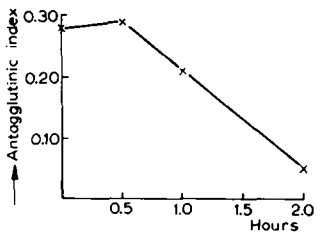

Fig. 7. Change in biological activity of antagglutin in a watery extract of cervical secretion by oxidation (oxygen in presence of colloidal platinum at room temperature).

thioglycolate. Nevertheless, the isolation of antagglutin from follicle fluid treated in this way gives an active product when tested in the reduced form.

The biological test method has been used in the present as well as in earlier investigations for the demonstration of antagglutinic activity in samples of female origin[6,7,14]. It was therefore considered important to obtain some knowledge about the relationship between the concentration of the female antagglutin and its biological effect. This was done by making series of dilutions of specimens containing antagglutin, and determining their antiagglutinic index. Fig. 8 shows the result for antagglutin samples purified on quartz columns. Contrary to the observations on purified male antagglutin, the range exhibiting proportionality between concentration and biological activity is very narrow. Above this range an increase in antagglutin concentration does not produce any further rise in the AAI. Besides, there seems to be always a critical concentration below which no antagglutinic activity takes place.

Fig. 9 shows a corresponding experiment with extracts of human cervical secretion from the 16th day of the cycle. The samples having a volume of about

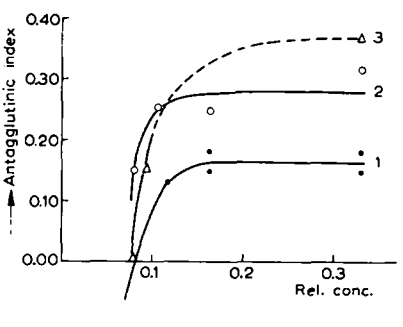

Fig. 8. Biological activity of samples of chromatographically isolated antagglutin plotted against relative concentration of purified substance. Curves 1 and 3 refer to the same material tested on two different samples of spermatozoa. The reduction of the antagglutin was effected by a surplus of thioglucolate.

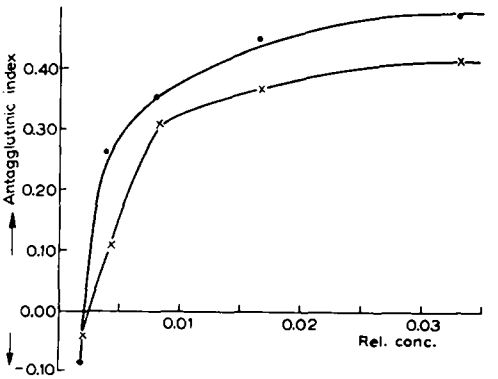

Fig. 9. Biological activity of two specimens of human cervical secretion from the 16th day of the cycle plotted against relative concentration, the latter expressed as parts of secretion per unit volume of tested extract. The reduction of the antagglutin was effected by a surplus of thioglucolate.

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0.025 ml were extracted twice with 5.0 ml redistilled water, the pooled extracts evaporated to dryness, and the residues dissolved in 0.5 ml diluter. These extracts, already containing the antagglutin in a twentyfold dilution, were then further diluted so as to give a graded series of concentrations of antagglutin. The general trend of the curves is the same as of those in Fig. 8. However, the bend is less pronounced, and the asymptote on a somewhat higher level.

Whereas purified preparations of male antagglutin—if sufficiently concentrated—may give indices up to 0.80 the AAI-values obtained with female antagglutin are on the whole much lower. The highest value ever observed, AAI = 0.75, was obtained with extracts of oestrous mucus of the cow, and with extracts of precipitates obtained from follicle fluid with ammonium sulphate. Samples prepared by precipitating follicle fluid with ethanol and further purification with non-polar solvents (*cf.* p. 23) have given values up to 0.59. The AAI of samples of female antagglutin purified by chromatography on quartz never exceeded 0.50, and generally lay between 0.30 and 0.40. It further appears that the numerical value of the index is dependent upon some quality of the spermatozoa used as test material, since curves (1) and (3) in Fig. 8 were obtained with the same antagglutin preparation, but with different spermatozoa.

DISCUSSION

So far we have not had the opportunity of controlling the homogeneity of our preparations by appropriate methods. The degree of purity may thus be estimated only indirectly. The absence of phosphorus and sulphur in our preparations excludes to any large extent contaminations by compounds containing these elements.

The results of the analysis of nitrogen, sulphur, and phosphorus may be dealt with in the following way. From our ultra filtration experiments it appears that the molecular weight of the antagglutin does not exceed 4,000. By comparing the smallest possible percentage of these elements in the antagglutin molecule, *i.e.* one atom in a molecular weight of 4,000, with the amount found and referred to the organic part of the analysed sample it may be decided whether the elements in question are constituents of the antagglutin or not. As can be seen from Table I, the figures found are from at least 2.4 to 10 times smaller than those expected. Thus the female antagglutin does not contain any of the analysed elements.

TABLE I

Element	*Expected percentage on the assumption of one atom being contained in a mol. weight of max. 4,000*	*Percentage actually found*	*Percentage referred to the organic portion of sample*
Nitrogen	0.35	0.02	0.033
Sulphur	0.8	<0.2	<0.35
Phosphorus	0.77	<0.1	

On account of their insolubility in water most lipids are excluded as impurities. Phospholipids and sphingolipids, both soluble in water, have already been considered,

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being P- and N-containing compounds, respectively. Possible impurities which cannot be excluded so far are *e.g.* esters of steroids.

Compared with the male sperm antagglutin the female substance appears to be less complicated, since it contains no proteinic constituent. It thus corresponds more to the "active group" of the male antagglutin than to this substance itself. To what extent the female antagglutin is reversibly coupled in its natural environment to a specific compound of proteinic or other nature cannot be checked so far. The positive results (*cf.* p. 26) obtained with the procedure elaborated by LINDAHL, KIHLSTRÖM, AND ROSS[8] as a quantitative method for male antagglutin indicate that the same or a very similar residue occurs in female antagglutin. Although the reactions underlying this method are still not fully explored, investigations on its specificity[15] show that the carbocyclic residue of the male antagglutin is the part of the molecule that is involved. The similarity of this component of the two substances is further stressed by the fact that a derivative with the same absorption spectrum in ultraviolet as has been registered[5] for the slightly denaturated "active group" of the male antagglutin was detected after some special treatment of a sample of follicle fluid. The female antagglutin further differs from the male substance in containing no sulphuric acid residues. In concordance with this fact the female antagglutin does not show metachromasy under any conditions. Further, it does not seem to be electrically charged.

The only constituents of the female antagglutin recognized up to now are, besides the carbocyclic compound mentioned, sugar residues whose reducing groups are engaged in bonds (*cf.* p. 24).

The change in the rate of dissolution in water occurring on treatment with apolar or slightly polar organic solvents must in some way or other concern the carbocyclic residue of the antagglutin molecule. Similar observations have also been made on the isolated "active group" of the male sperm antagglutin[5]. The mechanism of this change is still unknown.

Like the male antagglutin, the female substance may also be reversibly oxidized and reduced (Fig. 6), whereby only the reduced form exerts biological activity. The site of oxido-reduction is most probably located on the cyclic constituent of the two substances. With regard to the functional importance of the reduced form it is of great interest to note that although the oxygen pressure in the oviducts (rabbits) is high enough to support aerobic metabolism[16] the oxido-reduction potential of the secretions is said to be rather low[17]. In keeping with this is the detection of ascorbic acid in the secretory cells of the tube mucosa in the woman[18] as well as in the cow[19]. Accordingly, the antagglutin of the normal human cervical secretion during the ovulation phase was found to be protected against oxidation (Fig. 7). It thus seems very probable that the appearance of biologically non-active antagglutin in some cases of sterility[6] depends upon inability to secret the protecting substances, *e.g.* ascorbic acid. The follicle fluid also contains ascorbic acid[20]. In addition, other components probably contribute to the protecting action.

From a biological point of view the curve relating biological activity to concentration of female antagglutin (Fig. 8) is rather surprising. All the curves studied indicate that only a relatively small fraction of the agglutinated spermatozoa is dispersed even when high concentrations are added. The most plausible explanation of this fact is the assumption that some other factor occurring already in the follicle

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fluid and possibly also produced by the tubes must co-operate with the female antagglutin to give it its full biological activity. The higher AAI values of the less purified samples obtained by salting out with ammonium sulphate or by fractionated extraction, compared with the lower ones obtained with samples chromatographed on quartz, give support to this assumption.

As long as we are dealing with the estimation of antagglutinic activities the variation in the quality of the sperm suspension used as test object certainly is a source of error (*cf.* Fig. 8). This has not been systematically investigated so far. From the results imparted here it appears, furthermore, that the biological test method may not be used in its present form for estimations of quantities of antagglutin, especially not when purified samples are examined. However, when working with human cervical mucus the antagglutin concentration is generally so low that we are on the slope of the activity/concentration curve (Fig. 9). Nevertheless, there is no real proportionality between indices and amount of antagglutin since this is not a straight line.

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SUMMARY

1. Female sperm antagglutin has been isolated from the follicle fluid of the cow by adsorption on a column of quartz powder and fractionated elution with water saturated with carbon dioxide.

2. The substance, which is not a proteid like the male equivalent, is free from nitrogen, sulphur, and phosphorus. It contains non-reducing sugars and a carbocyclic residue, showing similarities to tocopherols and present also in male antagglutin.

3. The female antagglutin is very soluble in water, soluble in methanol and ethanol, and slightly soluble in ethyl ether, acetone, and carbon tetrachloride.

4. The female antagglutin gives positive reaction with the procedure elaborated by LINDAHL, KIHLSTRÖM, AND ROSS (1957) for the quantitative determination of male antagglutin. Applied to human cervical mucus, this reaction is positive during the ovulation phase, and negative in the later part of the cycle.

5. The biological activity referred to concentration shows an asymptotic course with lower values of the asymptote for purified antagglutin than for cruder preparations, *e.g.* cervical mucus. The antagglutin is protected against oxidation in the follicle fluid and the cervical mucus.

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A NEW HOMOGENIZER FOR THE ISOLATION OF NUCLEI IN CONCENTRATED GLYCERINE

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Our research into the protein content of the nuclei of beef pancreas, for which a large quantity of nuclei is needed, prompted us to design a homogenizer, capable of liberating the nuclei from several whole glands in a short time.

To reduce as much as possible the loss of protein from the nuclei during the isolation, we chose the medium described by SCHNEIDER[1], *i.e.* 70% glycerine, with the addition of substances which preserve the morphology of the isolated nuclei.

The homogenizer we needed had to fulfill three requirements. Firstly, it should allow continuous operation, since the working up of many small portions is very time-consuming. This consideration rules out the use of the classical POTTER-ELVEHJEM homogenizer[2] and related designs (DOUNCE *et al.*[3] and others). Secondly, the degree of homogenization should not depend on manual skill, since this tends to make the results poorly reproducible. Again, the Potter-Elvehjem and similar types do not meet this requirement. In the third place, the homogenizer must be suitable for the viscous 70% glycerine medium.

The only homogenizers, which to a certain extent meet these requirements, are the Waring blendor and the apparatus proposed by LANG AND SIEBERT[4]. Even though the Waring blendor does not allow a continuous procedure, the quantities it can accomodate are sufficiently large. This apparatus, however, destroys nuclei as well as the other elements and thus causes a large loss of nuclei.

The instrument of LANG AND SIEBERT consists of a cone, rotating within a mantle of the same shape. The suspension to be homogenized is forced by gravity through a narrow slit between the two conical surfaces. This homogenizer, though made for

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