STUDIES ON FERTILITY

1957

Including papers read at the
Conference of the Society for the Study of Fertility
Exeter, 1957

Being Volume IX of the Proceedings of the Society

Edited by

R. G. HARRISON
M.A., D.M.
Derby Professor of Anatomy in the University of Liverpool

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PREFACE

The publication of an annual volume by the Society for the Study of Fertility serves many purposes apart from acting as a medium for distribution of the Society's Proceedings amongst its own members. By making the papers read at its annual conference generally available, and publishing research work from investigators outside the Society's membership at home and overseas, dissemination of the results of important investigations from the wide field of reproductive physiology is ensured. To this end the present volume is broadly representative, since it contains papers related to human fertility and that of experimental animals, both male and female. Papers are presented from many specialist interests, including anatomy, veterinary physiology, experimental biology, genetics, animal breeding, endocrinology, reproductive biochemistry, obstetrics and gynaecology. These activities roughly reflect the wide membership of the Society for the Study of Fertility, and demonstrate the opportunities available at the annual conference for valuable discussion between workers in the various disciplines.

An innovation in this year's volume is the publication of the first lecture given under the auspices of the Oliver Bird Trust. This Trust has as its object the advancement of knowledge of methods of fertility control with special reference to the promotion and provision of facilities for tests and clinical trials of systemic methods of contraception as they become available, and in the meantime for the improvement of locally acting spermicides. It is therefore most appropriate that the first Oliver Bird Lecture should have been devoted to the biochemical characteristics of spermicides, and that no more appropriate lecturer in this field could be found than Dr. Mann whose researches have contributed so markedly to our knowledge of the biochemistry of semen.

The character of the chapters in this volume is identical with that of previous editions. Most of the papers published here were read at the Society's Annual Conference in 1957, but several have been published without previous delivery at the Conference. Each consists of a scientific paper resulting from original investigation. No paper is accepted which has been printed elsewhere, but reviews of previously published work of high quality are occasionally received. With these reservations, the Editor would be pleased to consider any manuscript for inclusion in future editions of this book. I am again indebted to Mr. Per Saugman and Mrs. J. M. Green for their advice and help in the publication of this volume, and to Miss M. R. Crowther and Miss M. J. Hoey for their assistance in the preparation of the indices.

The Society is again most mindful of the financial assistance it has received from Ciba Laboratories Ltd. which has assisted the publication of this volume.

R. G. HARRISON

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I

BIOCHEMICAL BASIS OF SPERMICIDAL ACTIVITY

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It is by no means a simple matter to define the biochemical basis of spermicidal activity, the main difficulty being to distinguish and to decide between the biochemical change which is the immediate result of the spermicidal action, and the secondary effects which develop at a later stage. In this respect, the position resembles somewhat the present situation in the field of antibiotics. There, too, we are confronted with the dilemma of how to disentangle the biochemical processes which are a direct outcome of bacteriostatic or bactericidal activity, and the various after- arise from the primary damage to the microbial cel etween the spermicidal agents and antibioti ppear to a superficial observer, the importar two classes of substances being that whilst, in general, a their most potent in an environment favourable to cellular growth and multiplication, the spermicidal agents are supposed to exert their full effect in reactions with cells which neither grow nor multiply.

This inherent inability of the spermatozoon to grow or to undergo cellular division not only sets apart the sperm cell as an extraordinary experimental object, but it is an aspect which must never be left out of consideration concerning the mechanism underlying the action of spermicidal agents. Of equal importance is the fact that in semen the spermatozoa are freely suspended in a liquid medium, the seminal plasma, each leading a life of its own as it were, so that in effect, the semen represents a suspension of cells resembling more a culture of microorganisms in a nutrient medium than other animal tissues which depend upon a complicated supply of nutrient material via the blood capillaries. A further remarkable and important feature which largely determines the behaviour of spermatozoa not only towards spermicidal agents but towards extraneous agents in general, is their exceptional

permeability. This property of the sperm cells explains the velocity with which enzymes located within the spermatozoa are capable of reacting with added substrates, and also the ease with which large protein molecules, such as for instance cytochrome *c* or hyaluronidase, detach themselves from the sperm structure and leak out into the surrounding medium. The reason for this striking sperm permeability is the peculiar threadlike structure of the sperm cell which makes possible various rapid exchange reactions between the spermatozoa and their surrounding medium, whether this be the seminal plasma or a composite artificial pabulum used for semen storage. The filiform character of the sperm cell is particularly evident in the shape of the midpiece-tail portion, and it is this portion of the cell which provides the site for most of the known enzymic activities, including the glycolytic system and the cytochrome system.

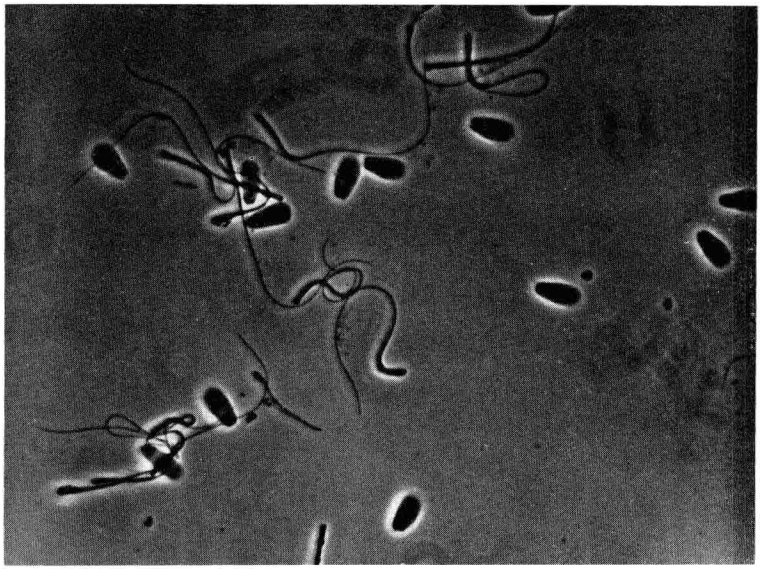

FIG. 1. *Spermatozoa with heads and midpiece-tails detached, in the semen from a sterile Guernsey bull.* By subjecting the semen to low-speed fractional centrifugation it was possible to obtain a heavier fraction, consisting of immobile sperm-heads, and a lighter fraction, consisting of head-free and yet perfectly motile midpiece-tails.

Above all other considerations, however, one which deserves special notice concerns the fact that Nature has endowed spermatozoa with two highly specialized biological functions, fertilizing capacity and motility, which not only set them apart from all other cells, but which at the same

time provide the investigator with two clearly defined and specific biological criteria of sperm activity, which he can utilize in the study of the efficacy of spermicidal agents. In the past, investigators often took sperm motility as the sole criterion for assessing the potency of spermicidal agents, but it is important to bear in mind that motility and ability to fertilize by no means always coincide. By way of an interesting example one might describe the results of an experiment carried out a little while ago with a Guernsey bull, in the semen of which practically all spermatozoa were in a 'disintegrated' state, that is, with heads separated from the midpiece-tail portions; the characteristic features observed on microscopical examination of the semen produced by this bull are illustrated in Fig. 1. Although separated from the heads, the midpiece-tail portions present in this semen were nevertheless perfectly motile. Needless to add, in spite of this motility, the semen of this bull

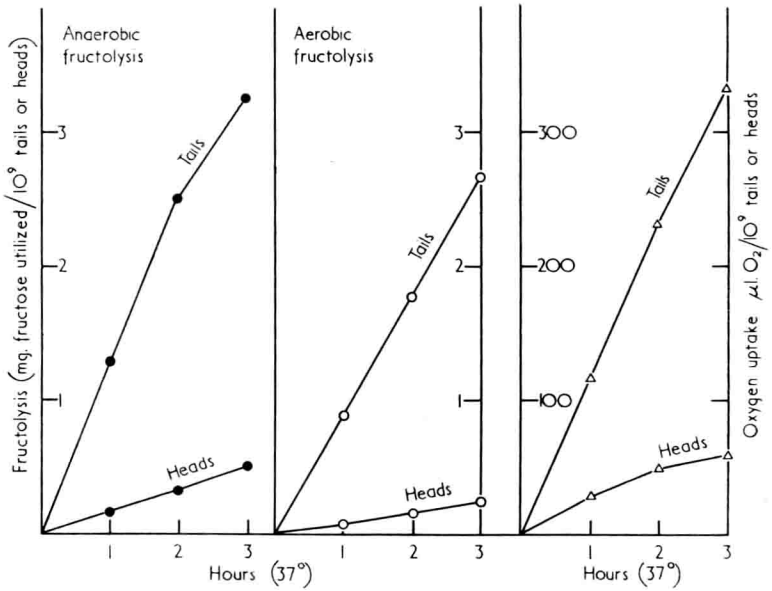

FIG. 2. *Fructolysis and respiration in sperm-heads (immotile) and midpiece-tails (motile).* The heads and tails were obtained by slow-speed fractional centrifugation from a Guernsey bull whose ejaculates consist entirely of disintegrated spermatozoa. After mixing with Ringer-solution, the suspensions of heads and midpiece-tails were diluted so as to have the same density, and examined for anaerobic fructolysis (in N_2), aerobic fructolysis (in air) and respiration (in air), at 37° C.

was completely infertile. By subjecting such semen to low-speed centrifugation it has been possible to obtain two distinct fractions, a heavier

one, made up of immobile sperm-heads, and a top fraction, consisting of a suspension of head-free, and yet perfectly mobile, tails. These two fractions have been examined separately as regards fructolysis and respiration. It can be seen from Fig. 2 that fructolysis and respiration are mainly confined to the tails, and that metabolically the 'head-fraction' is much less active than the 'tail-fraction'. Moreover, most of the cytochrome pigments and a great portion of phospholipid is also to be found in the 'tail-fraction'; similarly the adenosinetriphosphatase activity of the spermatozoa is within the tails, and not in the heads. On the other hand, deoxyribonucleic acid is confined to the sperm-heads, ribonucleic acid being absent from both heads and tails (Table I).

TABLE I

DISTRIBUTION OF DRY MATTER, NITROGEN AND CERTAIN PHOSPHORUS COMPOUNDS BETWEEN THE SPERM-HEADS AND SPERM-TAILS

Results are expressed in mg./100 ml. of semen obtained from the Guernsey bull whose ejaculates consisted entirely of free sperm-heads and free midpiece-tails. Before testing, the heads and tails were washed with Ringer solution

	Heads	*Tails*	*Together*
Dry weight	684	839	1523
Non-protein nitrogen	5.6	17.3	22.9
Inorganic phosphorus	0.8	1.0	1.8
Acid-soluble phosphorus	0.7	1.8	2.5
Lipid-phosphorus	0.9	2.3	3.2
Residual phosphorus	19.3	0.5	19.8
Ribonucleic acid	not detectable		

The principal feature of spermicidal activity as displayed in the inhibition of sperm motility has been recognized and utilized for experimental purposes already by the pioneers in sperm physiology: Leeuwenhoek (1678), Spallanzani (1776), Prévost and Dumas (1824) and Donné (1837). Certain outstandingly important investigations date from the last century, namely, those of Quatrefages in France, Newport in England and Kölliker in Germany. Quatrefages (1850, 1853) described in great detail the toxicity to spermatozoa of salts of heavy metals such as copper, mercury and lead. Newport (1853) observed the 'narcotizing' influence of chloroform vapours on the amphibian spermatozoa, and stated 'that the spermatozoon does not impregnate when entirely deprived of its power of motion by narcotization and disenabled to penetrate into the envelopes of the egg'. Kölliker's experiments embodied in his paper on 'Physiologische Studien über die Samenflüssigkeit' (1856) included numerous findings on the dilution effect and on the toxic action of various inorganic ions as well as certain organic substances. He also noted that cyanide is not always an inhibitor of sperm motility. This somewhat unexpected behaviour of cyanide arises, as we

now know, from the fact that cyanide can, if used in appropriate concentrations, inhibit cellular respiration without affecting glycolysis: spermatozoa are capable of maintaining their motility at the expense of metabolic energy derived from either respiration or glycolysis, and it is therefore not surprising that the elimination of only one of these two metabolic processes does not necessarily involve a complete sperm standstill.

The present century has witnessed several important advances in the field of research on spermicidal substances. Among those which deserve special mention are the early investigations by Günther (1907) who described the inhibition of sperm motility by phenolic substances, fluoride and hydrogen peroxide. Later on Gellhorn (1920, 1922, 1924, 1931) studied the effect of variations in hydrogen ion concentration, tonicity and ionic composition of media; Schlenk (1933) demonstrated that certain spermicidal substances, when added to semen, produce a sudden short-lived outburst of increased sperm activity, something akin to a 'terminal convulsion' ('Todeszuckung') which precedes the ultimate loss of mobility; Henle and Zittle (1941) made a similar observation on bovine spermatozoa, pointing out that the addition of gramicidine increases the O_2-uptake before suppressing it; Lardy and Phillips (1943) described the influence of various metabolic inhibitors including glyceraldehyde, maleate and arsenite; MacLeod (1946, 1951) proved that the inhibition of sperm motility and metabolism by organic arsenicals can be overcome by 1 : 2 : 3-trithiolpropane, and that the inhibition by cupric ions can be counteracted effectively by the addition of cysteine or glutathione; and White (1955a, b; 1956) demonstrated a synergistic inhibitory effect of metals and certain chelating agents.

A great deal of information concerning the chemical nature of the various spermicidal agents has been summarized by Baker (1935) in his book on *The Chemical Control of Conception* and by Millman (1952), Pirie (1952), Henshaw (1953), Davidson (1953) and Gamble (1953, 1957a, b) in their respective articles. Most of the work described there, however, was concerned mainly with the elaboration of methods for assessing the spermicidal power and with the application of spermicidal agents to contraception, and not with the biochemical basis of spermicidal activity, i.e. the elucidation of the metabolic processes responsible for the action of spermicidal agents on the spermatozoa.

Some time ago, in a communication to the Society for the Study of Fertility, I made an attempt to classify the spermicidal agents according to their mode of action (Mann, 1954a) and tonight I would like to expand further this topic.

The first point which I wish to make is that spermicidal agents differ in their action on spermatozoa. Our own experiments, and those of several other investigators engaged in biochemical studies of semen,

point to the existence of several distinct biochemical mechanisms whereby spermicidal agents act on spermatozoa. Each of these groups has its own characteristics, but there are some spermicidal substances which exhibit peculiarities of more than one group. The second point worth considering is that some spermicidal agents act on the spermatozoa instantaneously, whilst others develop their activity gradually though ultimately they may produce a high level of inhibition. It is therefore essential to assay the spermicidal activity of any given chemical substance at different time-intervals, not relying merely on a single-point determination. Another set of facts which needs stressing is that while some substances inhibit sperm motility and metabolism in a permanent — that is irreversible — manner, others, e.g. certain enzyme inhibitors, act reversibly by producing an inhibition which may be strong or even complete at first, but which diminishes when the inhibitory agent has been removed. Strictly speaking, only the irreversibly acting substances deserve to be called *spermicidal* agents, in distinction to the reversibly acting inhibitors which one might designate as *spermiostatic*. This aspect merits particular attention when explanation is sought for the behaviour of substances which, though they are known to be most effective *in vitro*, fail to exert a similarly strong inhibition under conditions *in vivo*. It is not to be expected that a reversibly acting inhibitor would maintain its potency for long in the female reproductive tract, where owing to the dispersal of semen, dilution with secretions, and progressive removal through absorption, the actual concentration of the inhibitor is continuously declining. There is one other closely related aspect which needs mentioning, as it might be of practical value. It concerns the fact that widely divergent results may be attained with a given substance according to a particular set of experimental conditions. I should like to quote three examples to support this statement. One is derived from a study by Bishop and Mathews (1952) who investigated the sperm-immobilizing action of *triphenyltetrazolium chloride*. They found that while washed mammalian spermatozoa in a glucose-free medium are immobilized completely by 0.0001 M of this compound, a 200 times higher concentration, i.e. 0.02 M, is ineffective towards the same sperm when it is suspended in a glucose-containing medium. The second example comes from our own experiments on the spermicidal activity of *cetyltrimethylammonium bromide*. It was found that in the presence of seminal plasma or of cells other than spermatozoa (e.g. yeast), much more cetyltrimethylammonium bromide is required in order to immobilize spermatozoa than when it is allowed to act upon sperm suspensions alone. This is due to the fact that this detergent, like so many other spermicidal agents, interacts not only with the spermatozoa themselves, but is also taken up, and thus in a sense neutralized, by either the seminal plasma or the other (added) cells. We have yet

another example in the experiments of Dunn, Lepard, Murphy and Garrett (1942) on the effect of *Amphyl* (a disinfectant consisting of a mixture of *p*-chloro-*m*-dimethylhydroxybenzene, amylhydroxybenzene and soap); this preparation was found to inhibit sperm motility when added to bull semen directly but it actually prolonged sperm survival when added to a mixture of bull semen and egg yolk diluent.

On the basis of their biochemical activity it may be convenient to group the spermicidal agents and sperm inhibitors into several distinct classes; the four main groups are: electrolytes, enzymic inhibitors, sulphydryl-binding substances, and surface-active agents.

ELECTROLYTES

The phenomenon of sperm inhibition brought about by hypotonic or hypertonic solutions has a long history behind it, reaching as far back as Leeuwenhoek's original observation that dilution of dog semen

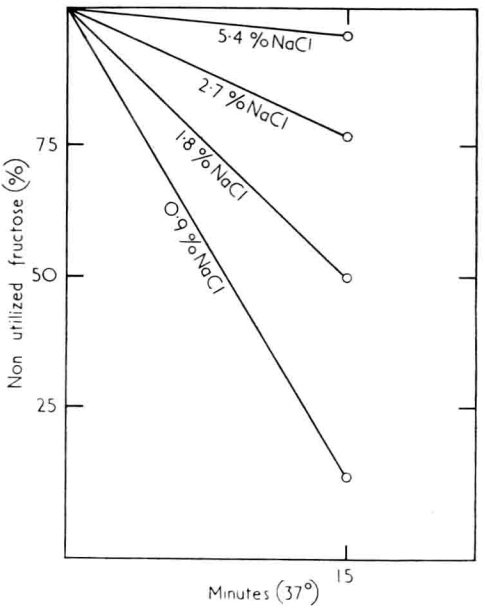

FIG. 3. *Effect of hypertonic solutions of sodium chloride on sperm fructolysis.* Ram sperm diluted with Ringer-phosphate-fructose solution. NaCl was added to give the final concentration indicated in the figure.

with rain water deprives the canine 'animalculi' of motion (Leeuwenhoek, 1678), and Kölliker's equally significant demonstration that spermatozoa rendered motionless by dilution with water can be revived

by prompt addition of salts (Kölliker, 1856). A survey of the various effects associated with changes in tonicity of media was made by Emmens (1948) and Blackshaw and Emmens (1951) as well as in the author's monograph on the *Biochemistry of Semen* (Mann, 1954b). An interesting recent development, however, which deserves mention is the exploitation of the spermicidal activity of concentrated solutions of sodium chloride incorporated in so-called salt-jellies, which have been investigated by Gamble (1953, 1957a, b) as potential chemical contraceptives. We have studied the effect of hypertonic solutions of sodium chloride on the metabolism of ram spermatozoa and found that fructolysis is abolished completely in the presence of 5% NaCl (Fig. 3).

ENZYMIC INHIBITORS

Here belong chemical compounds which exert their sperm-inactivating property by inhibiting enzymes, more particularly those concerned in the intermediary metabolism.

The two oldest known members of this group are *cyanide* and *carbon monoxide*. Both these substances inhibit the respiration of spermatozoa by combining with an essential respiratory enzyme, the cytochrome oxidase. The immobilizing action of cyanide can best be demonstrated in aerobically incubated sperm suspensions from which the fructose-containing seminal plasma has been removed by centrifugation and washing. The respiration of such washed spermatozoa depends on the oxidation of endogenous (intracellular) substrate and is strongly inhibited by cyanide. When, however, the washed spermatozoa are treated with cyanide but shortly afterwards recombined with seminal plasma, then they regain their motility. This is due to the fact that by the addition of seminal plasma the spermatozoa have been supplied with an extracellular source of energy in the form of fructose. The utilization of fructose (sperm fructolysis) is much less sensitive to cyanide than respiration and consequently can provide, despite cyanide, the energy necessary for sperm motility. The action of carbon monoxide can be demonstrated not only by its strongly depressant effect on the oxygen uptake of spermatozoa but also spectroscopically. When carbon monoxide is passed through a sperm suspension it is possible to observe under the microspectroscope a characteristic change in the position of the so-called cytochrome a_3 band, due to the formation of the CO-cytochrome a_3 complex (Mann, 1945). This complex, however, is light-sensitive, and the inhibition of sperm motility and respiration by carbon monoxide can be reversed promptly and completely by exposing the gassed sperm suspension to strong light of a suitable wave-length (Rothschild, 1948a, b). An effect upon sperm motility and respiration, similar in nature to that exerted