

Samples without fluorochrome, in the dark: $k = 0.018 \text{ hr.}^{-1}$, $t_{1/2} = 38 \text{ hr.}$

Samples without fluorochrome, illuminated: $k = 0.078 \text{ hr.}^{-1}$, $t_{1/2} = 8.8 \text{ hr.}$

Samples with acridine-orange, in the dark: $k = 0.017 \text{ hr.}^{-1}$, $t_{1/2} = 40 \text{ hr.}$

Samples with acridine-orange, illuminated: $k = 0.43 \text{ hr.}^{-1}$, $t_{1/2} = 1.6 \text{ hr.}$

There is no significant difference in slope between the curves obtained for the samples with and without acridine-orange kept in darkness ($P > 0.90$). The slopes of both curves differ significantly from a horizontal line ($P < 0.001$).

The slope of the curve of the samples without acridine-orange, kept in darkness, differs significantly from that of the illuminated portions of the same samples ($0.02 < P < 0.05$), illumination increasing the rate of reduction of the number of spermatozoa.

The slope of the curve of the illuminated samples with acridine-orange is significantly steeper than that of the curves obtained for the samples kept in darkness ($P < 0.001$), and also than that of the curve obtained for the samples with acridine-orange kept in the dark.

These results confirm that the number of normally moving spermatozoa in diluted semen without the addition of fluorochrome decreases much faster under the influence of illumination than in darkness, in agreement with previous findings by Norman and Goldberg⁷, whose results, however, do not allow further analysis of the relationship since evaluations were performed at two time intervals only. The ratio of the slope constants in these two conditions is obviously dependent on the actual level of illumination employed. This is not the case with a comparison between the constants for the slopes obtained for the same illuminated samples with and without acridine-orange, since both are then exposed to the same light intensity. Whereas in darkness acridine-orange (1:50,000) did not have any demonstrable effect on the mean velocity and the number of normally moving sperms during a period of storage in a Dewar vessel with ice up to 30 hr., a big and deleterious effect in both respects occurs in the samples kept under illumination; this effect is enhanced considerably by photosensitization by acridine-orange.

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¹ Duijn, jun., C. van, *Nature*, **187**, 1006 (1960).

² Rikmenspoel, R., and Van Herpen, G., *Physica Medica Biol.*, **2**, 54 (1957).

³ Rikmenspoel, R., "Photoelectric and Cinematographic Measurements of the Motility of Bull Sperm Cells", thesis, Utrecht (1957).

⁴ Rikmenspoel, R., *J. Agric. Sci.*, **57**, 399 (1960).

⁵ Duijn, jun., C. van, and Rikmenspoel, R., *J. Agric. Sci.*, **54**, 300 (1960).

⁶ Rikmenspoel, R., and Duijn, jun., C. van, *Tijdschr. Diergeneesk.*, **85**, 1002 (1960).

⁷ Norman, Ch., and Goldberg, E., *Science*, **130**, 624 (1959).

Size of Particles Ingested by *Simulium* Larvæ

THE diet of the aquatic larval stages of the blackfly, *Simulium*, some adults of which transmit onchocerciasis to man and animals, has been described; but little information is available on the size of the particles ingested.

The size of particulate matter taken in by *Simulium* larvæ has been measured for four temperate species of *Simulium*.

S. ornatum was obtained from a small highly calcareous stream at Hawarden, Flintshire. This stream is heavily shaded by trees and slightly polluted by farm drainage.

S. ornatum, *S. reptans* and *S. variegatum* were collected from the River Ceirog at Chirk, Denbighshire. This river, a tributary of the Dee, is shaded by trees, is moderately calcareous, and is slightly polluted from cattle pasture.

S. tuberosum was obtained from the Hirnant, a soft-water hill stream at Bala, Merionethshire.

The contents of the midgut were separated from the gut tissue on a slide, and spread by gentle pressure on a coverslip. The longest and shortest axes of 45 randomly selected particles were measured in 5 last instar larvæ in each group, and in 5 earlier instar larvæ which were not identified, taken from the Ceirog.

The frequency distribution of particle sizes in each group was log normal, and the sizes in the individuals of the different species from the different rivers were similar.

(*S. ornatum*, Hawarden, $11.3 \pm 1.8\mu$, $6.6 \pm 1.7\mu$; *S. ornatum*, Ceirog, $13.2 \pm 2.1\mu$, $7.3 \pm 1.8\mu$; *S. reptans*, $15.1 \pm 2.0\mu$; $8.0 \pm 1.8\mu$; *S. variegatum*, $14.8 \pm 2.1\mu$, $7.8 \pm 1.9\mu$; *S. tuberosum*, $12.0 \pm 1.9\mu$, $7.7 \pm 1.9\mu$; unidentified early instars $14.0 \pm 1.7\mu$, $7.3 \pm 1.7\mu$.)

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BACTERIOLOGY

Chelating Agents for Selective Growth of Bacteria

THE use of pH buffers for stabilizing the pH of growth media has become common bacteriological practice. There do not appear to be any reports, however, of the similar use of chelating agents for controlling the concentration of metal ions as 'metal buffers'^{1,2}. With metal ions there is a more complex situation, of course, since there are few chelating agents selective for one particular metal. An observed effect on bacterial growth can, therefore, be due to simultaneous sequestration of several metal ions and this must be allowed for in designing experiments.

It has been found that, by suitable control of the concentration of magnesium ions, liquid and solid media can be devised for selection of coagulase-positive staphylococci in presence of coagulase-negative strains and of many commensal organisms. By calculation from the controlled pH and from the known stability constants of the chelating agents³ employed, it is found that the concentration-range over which an effect can be observed in the growth-rate of coagulase-positive staphylococci lies roughly between $M/1,000,000$ and $M/25,000$ free magnesium ions (or if expressed more conveniently in terms of the negative logarithm, from pMg 6.0 to 4.4). This accords with the value of $<M/250,000$ to $M/25,000$