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Taxonomic Position of the Genus *Selenomonas* (von Prowazek)

In a recent analytical review[1], Lessel and Breed have reiterated the conclusions of Boskamp[2] upon the bacterial nature of *Selenomonas palpitans* (Simons, *ex* Boskamp). Although they do not add materially to the information available, these authors state that *Selenomonas* clearly belongs to the family Spirillaceae.

I have studied the morphology both of *Selenomonas palpitans* from the guinea pig cæcum, and of *S. sputigena* from the human mouth, and am of the opinion that the nuclear structures, flagellation and mode of cell division are entirely unlike those of bacteria and reflect a much closer relationship with the protozoa.

The nucleus consists of a filament with expanded ends, one of which is connected by a rhizoplast to the blepharoplast, from which arises the flagellum, usually at the centre of the concave side of the crescentic cell. The nucleus and a portion of the neuromotor apparatus are embedded in deeply staining basophilic masses, in the form of two cones, point to point (Fig. 1,*b*). When the nucleus divides, a second rhizoplast appears, joining the blepharoplast to the free end of the nuclear filament. The blepharoplast divides, initiating division of the flagellum, and the division of the nucleus follows (Fig. 1,*c*). The cell then divides by constriction. This is entirely unlike any known scheme of nuclear division in bacteria[3].

The cell wall fails to stain in the characteristically bacterial manner with tannic acid and crystal violet, or with phosphomolybdic acid and methyl green[3,4], and the cell lacks the cross-walls which are found in

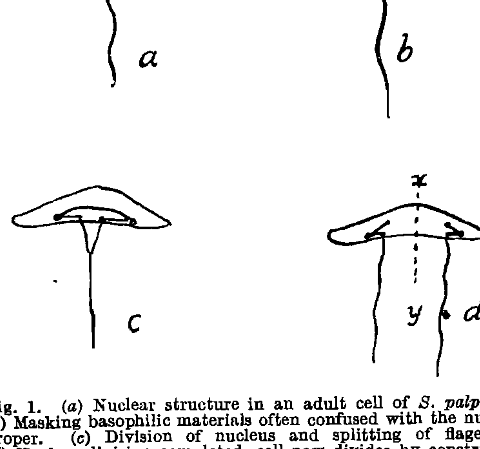

Fig. 1. (*a*) Nuclear structure in an adult cell of *S. palpitans*. (*b*) Masking basophilic materials often confused with the nucleus proper. (*c*) Division of nucleus and splitting of flagellum. (*d*) Nuclear division completed, cell now divides by constriction along *x*—*y*

many bacteria, including spirilla[3]. The flagellum, when compared with those of bacteria in the same field of the electron microscope, was entirely distinct in its appearance. It is composed of numerous tiny fibrils, like the flagella of protozoa, and unlike either the monofibrillar flagella of bacteria or the compound flagella of certain spirilla[3]. The flagellum of *Selenomonas* is also distinct from that of bacteria in that it can be demonstrated in the light-microscope by staining with Giemsa or simple dyes without previous mordanting. The frayed 'whip-tuft' appearance shown by Boskamp[2] and others is an artefact, due to unsuitable technique. The cell moves in an irregular tumbling course, which once more is more reminiscent of protozoa than of bacteria.

These findings indicate clearly that the affinities of the genus *Selenomonas* are with the Protozoa. The classification of Wenyon[5], which places it in the family Monadidae (Kent), is adequate to the present extent of our information.

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Paper Chromatographic and Biological Properties of Reserpine and Related Compounds

Little is known of the metabolism (in man and experimental animals) of the alkaloids of *Rauwolfia serpentina*. We are particularly interested in the reported antifertility effects of reserpine in rats[1] and the mechanisms of sedative action[2] of *Rauwolfia serpentina* alkaloids in schizophrenic patients. Reserpine is known[3] to be hydrolysed *in vivo* to methyl reserpate and presumably trimethoxybenzoic acid. The latter compound has been shown[4] to influence certain enzyme systems *in vitro* which are of importance in relation to the mode of action of œstrogens on the uterus. Using a fertility performance test, we have studied the effects of the administration to male and female rats of daily oral doses of 80–100 mgm. of sodium trimethoxybenzoate given over a period of ten weeks. Since no alteration in fertility was observed, we conclude that reserpine itself or some polycyclic metabolite is responsible for the observations of previous workers[1].

We recommend the following paper chromatographic procedures as a means of checking the purity of commercial samples of *Rauwolfia* alkaloids and determining the composition of complex mixtures of the alkaloids used for therapeutic purposes and in pharmacological tests. At the time of writing, we are unaware of any detailed reports on paper chromatography of *Rauwolfia* alkaloids. The methods introduced by us employ Whatman paper No. 542 and the following developing solvent systems:

(1) '*Single phase*' *systems* (no pre-equilibration, capillary ascent technique employed).

(*a*) 10 per cent (v/v) acetic acid in aqueous 5 per cent sodium acetate is shaken with *n*-butyl ether, added in small portions, until saturation of the aqueous phase is just reached. The clear aqueous phase is used.

(*b*), (*c*) and (*d*), similar to (*a*) but using tertiary amyl alcohol, *sec.*-butyl alcohol or methyl*iso*butylketone instead of *n*-butyl ether. (*d*) was particularly useful for slow-moving components in (*a*). Similar solvents have been used previously for urinary phenols and œstriol[5].

(*e*) Xylene 200, methanol 75, methyl*iso*butylketone 25.

(2) *Partition chromatography* (pre-equilibration required).

(*a*) *n*-Amyl alcohol 200, water 180, acetic acid 20.

(*b*) *n*-Hexyl ether 200, methyl*iso*butylketone 50, acetic acid 20, water 180.

Two-dimensional paper chromatography of a commercial concentrate of reserpine and other alkaloids from *Rauwolfia serpentina* revealed twenty-four fluorescent components without using any spray reagents. Suitable combinations of solvents were (2*b*) followed by (1*a*) or (1*b*), or (1*e*) followed by (1*a*).

The following tests may be used for locating the compounds: (i) Fluorescence in ultra-violet light (Wood's glass filter). (ii) Fluorescence after streaking or spotting the paper with 3 per cent sodium nitroprusside in 50 per cent trichloracetic acid (cf. ref. 3). (iii) Fluorescence 30 min. after spotting the paper with microdroplets of 90 per cent sulphuric acid and later spraying with 9 per cent sodium carbonate. Intense blue, green or greenish-blue fluorescent spots are obtained in the alkaloid zones. (iv) Tests (ii) and (iii) followed by spraying with alkaline diazotized I.C.I. 5091 [5]—pink colours. Methyl reserpate and ajmaline are particularly intense. (v) Ehrlich's dimethylaminobenzaldehyde reagent; ajmaline-blue spot which is intensified by the reagent used in (ii). (vi) Bismuth iodide – iodine in ethyl acetate, acetic acid followed by 0·5 per cent sodium dithionite to remove the orange background. Several compounds give salmon-coloured spots; but the sensitivity is not high.

Tests (i) to (iv) are used together as a routine on single chromatograms.

Solvent system (1*a*) gave the best results and tests (ii) and (iii) gave positive reactions with less than 1 μgm. amounts of reserpine and rescinnamine.

The following relative R_F values were obtained using solvent (1*a*): ajmaline 0·76, deserpidine 0·41, methyl reserpate 0·52 principal spot, and 0·76 as well as two other minor components, rauwolscine 0·63, reserpine 0·34, rescinnamine 0·26, serpentine 0·5, yohimbine 0·58, new yohimbine 0·62. With solvent (1*b*) R_F values were: ajmaline 0·75, ajmalicine 0·5, canescine 0·59, corynanthine 0·64, reserpine 0·43, and impurity 0·55, rescinnamine 0·31, and impurity 0·41, serpentine 0·5.

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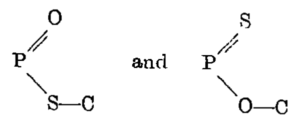
A General Method for locating the Spots on a Paper Chromatogram of Tertiary Alkyl Phosphates, Thiolophosphates and Thionophosphates

There is no method described in the literature that is generally satisfactory for rendering these compounds visible in paper chromatography. Hanes and Isherwood[1] describe a method for phosphoramides, for example, 'Schradan'. This method fails with many compounds containing



In the case of 'Systox', about 50 μgm. gives positive results when treated with alkali for a period of up to 70 hr.

J. W. Cook[2] reported a new spot test for some organo-phosphorus compounds containing sulphur ('Systox' and its isomers). In this method the chromatogram is first treated with N-bromosuccinimide and then with fluorescein. The former reacts with the sulphur-containing organo-phosphorus compound. The remaining area of the paper contains free N-bromosuccinimide which afterwards reacts with fluorescein, forming a non-fluorescent compound. Exposing the chromatogram to ultra-violet light renders the organo-phosphorus compound visible as a fluorescent spot. Only when the proper amounts of N-bromosuccinimide and fluorescein are used is the bromine completely absorbed by the spot, which is then located by the fluorescence of fluorescein. This method has therefore the disadvantage that a controlled amount of bromine and fluorescein has to be used. If a quantity of N-bromosuccinimide greater than is needed to brominate the compound is used, the excess will brominate the fluorescein as in the background, and thus mask the spot. Similarly, an excess of fluorescein will mask the spot through fluorescence of the background.

If there is more than one spot and the quantities of substance in the spots differ, it follows that it is impossible to adjust the amounts of reagents to reveal them all and give an indication of their concentration in any one chromatogram.

It was found that all organo-phosphorus compounds containing sulphur so far investigated are, after treatment with N-bromosuccinimide (dissolved in acetone), much easier to hydrolyse. Treatment with ammonium molybdate solution as used by Hanes and Isherwood[1] formed blue spots after 10–20 min. exposure to ultra-violet light. The method is very sensitive; less than 7 μgm. may be detected as very clear, distinct spots. Details of this work will be published later.

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