Effectiveness of oral treatment with ketoconazole in artificial mycoses.

Animal species	Infection site	Treatment mode*	Number of animals with complete cure versus total number of infected animals at stated doses (mg/kg)					
			0	2.5	5	10	20	40
Candida albicans								
Turkey	Crop	prop.	0/18	16/22	13/14	-	-	-
Turkey	Crop	ther. 10**	0/28	10/19	18/19	10/10	-	-
Rat	Vagina	prop.	0/106	21/26	22/22	-	-	-
Rat	Vagina	ther. 5**	0/17	1/5	27/30	18/18	-	-
Rat	Vagina	ther. 3**	0/62	-	3/6	83/85	6/6	-
Guinea-pig	Skin	prop. a)	0/22	10/17	-	20/22	-	-
Guinea-pig	i.v.	prop. a)	0/15	4/6	6/11	15/15	-	-
Chicken	i.v.	prop.	0/18	3/6	6/6	12/12	-	-
Trichophyton mentagrophytes								
Guinea-pig	Skin	prop. a)	0/12	-	1/12	5/11	10/10	-
Guinea-pig	Skin	ther. b)	0/10	-	-	5/11	12/12	-
Microsporum canis								
Guinea-pig	Skin	prop. a)	0/20	-	-	3/22	24/24	-
Guinea-pig	Skin	ther. b)	0/10	-	-	6/12	6/12	11/12

* prop.: prophylactic treatment: starts either day of infection or 24 h prior to infection a); ther.: therapeutic treatment: starts 72 h after infection or 48 h after infection b). ** The duration of treatment was 14 days except when indicated.

Oral doses of 5 or 10 mg/kg b.wt and given for 14 days are 100% effective against systemic candidosis in chickens and guinea-pigs, respectively. The disease, induced in adult male guinea-pigs by injecting $8 \cdot 10^6$ cells of *C. albicans* (strain B 2630) in a lateral vein of the penis, resulted in the death of at least 50% of the control animals during the 2nd week after infection, with all survivors showing generalized deep candidosis and pronounced mucocutaneous candidosis. Systemic candidosis in young Hysex chickens was induced by i.v. administration of 10^8 cells of the same *C. albicans* strain.

The oral activity of ketoconazole against dermatophytoses was evaluated in guinea-pigs artificially infected on the back with either *Microsporum canis* or with *Trichophyton mentagrophytes*[3], it was found to be excellent both prophylactically and therapeutically at dose levels of 10–40 mg/kg b.wt given for 14 days.

The details of these and other investigations to delineate the compound's in vivo and in vitro chemotherapeutic spectrum of activity, its safety, mechanism of action, metabolism, etc. will be published in subsequent papers. Clinical studies are underway.

1 This work was supported by the 'Instituut tot Aanmoediging van het Wetenschappelijk Onderzoek in Nijverheid en Landbouw'.

2 J. Heeres, L.J.J. Backx, J.H. Mostmans and J. Van Cutsem, J. med. Chem., submitted.
3 E.F. Godefroi, J. Heeres, J. Van Cutsem and P.A.J. Janssen, J. med. Chem. *12*, 784 (1969).
4 J. Van Cutsem and D. Thienpont, Chemotherapy *17*, 392 (1972).
5 J. Van Cutsem and D. Thienpont, Sabouraudia *9*, 17 (1971).

Tsetse fly feeding sites (Diptera: Glossinidae)

E.J. Popham, M. Abdillahi and D. Lavin[1]

Department of Biology, The University of Salford, Salford M5 4WT (England), 31 August 1978

Summary. Tsetse flies are attracted to radiant heat, but do not use mammalian skin energy flux differentials to find sites from which blood is obtainable. Tsetse flies only feed where the pelt is less than 5 mm thick.

Tsetse flies are attracted to hosts from a distance by visual and olfactory cues[2–8]. But at short range it would be interesting to know if tsetse flies are attracted by radiant heat and use mammalian skin energy flux differentials to find sites which could provide a blood meal.

Materials and methods. All experiments were performed at 25 °C, 80% relative humidity and under an illumination of 120±20 lux. Live pupae of *Glossina morsitans* Westwood and *G. austeni* Newstead were obtained from the Tsetse Research Laboratories, Langford, Bristol. Pilot experiments and stereo electron micrographs of tsetse fly mouthparts failed to reveal any sex feeding habit differences and both sexes have been used in all experiments. Radiant energy flux of mammalian skin was measured with a Moll thermopile held 2.5 cm from the skin and the output recorded on a Solatron Digital Voltmeter. The thermopile gave 40±5% mV/W/cm^2, with a response time of 2 sec. A mean of 10 readings taken as the final result. A linear response with the amount of energy flux radiated was obtained[9]. Measurements were made on guinea-pig and rabbit, and the results given in table 2. The reactions of flies to radiant heat was tested using an 800 ml conical flask painted matt black, placed against either end of a cage 76×48×48 cm formed from a metal frame covered with black nylon netting. The flask was filled with hot water at 30–50 °C and the control flask at 25 °C. Tests showed the nylon netting prevented convection currents forming within the cage. In each experiment 10 unfed teneral flies were introduced into the cage and subjected to 12 h light and 12 h darkness for 2 days. At the start of the 3rd day, the hot flask was placed

in position, the light switched on and the number of flies going to each flask in the ensuing 15 min recorded. This preexperimental procedure was adopted because *G. morsitans*[10] and *G. austeni*[4] feed from dawn onwards. The results of the experiments are summarized in table 1. Batches of up to 134 unfed teneral flies were liberated into the cage described above containing either an anaesthetized rabbit or guinea-pig and the sites where the flies fed or failed to feed were recorded together with the pelt thickness at each site. The mutual reactions of tsetse flies and small mammals were investigated using 10 3-day-old unfed flies in a cage described above containing either 2 common lizards, or 2 mice or 2 gerbils or 2 rats or a guinea-pig or a rabbit. The behaviour of flies and host were recorded. An area 8×8 cm was shaved on one side of a guinea-pig and the opposite side used as a control. 5 unfed teneral flies were placed in a tube with the open end covered with nylon netting. The open end was held against the animal and the time taken for the flies to feed recorded.

Results and discussion. Table 1 shows that tsetse flies are attracted to radiant heat, but table 2 shows that in rabbit and guinea-pig there is no close correlation between energy flux and pelt thickness. S.c. fat and bone apparently reduce heat loss in some skin areas. Tsetse flies flew straight from the netting to the hot flask and attempted to probe. Table 1 shows that as the temperature of the hot flask rose from 25 to 40 °C, the proportion of flies attracted to it increased but declined above this temperature. This suggests flies were attracted by radiant heat to the hot flask. Flies were only attracted providing they had previously been subjected to a 12 h alternation of light and darkness over at least 48 h, and would not do so if this cycle were interrupted. When the flies were admitted to the cage containing anaesthetized rabbits or guinea-pigs *G. morsitans* settled on the netting and then flew over the mammal. *G. austeni* made no such preliminary exploratory flight. The flies were initially attracted to all parts of the body but only those that settled on the nostrils, eyelids, ears, between the toes and round the anus were able to gorge. These skin areas have either short or no hair and a pelt thickness of less than 1–2 mm. Flies settling elsewhere tunnelled between the hairs, failed to reach the skin, withdrew and attempted to feed elsewhere. Flies allowed to feed on shaved and unshaven abdominal areas of guinea-pigs had 90% of *G. morsitans* feed on the 1st day taking an average of 2.5 min to do so. Only 25% fed on day 27 taking 12.5 min to feed, the pelt thickness being 3.8 mm. None fed on the 38th day, when the pelt was 5 mm thick. 70% of *G. austeni* fed on day 1 and took a mean time

Table 1. Number of flies of *G. morsitans* and *G. austeni* attracted to the hot and cold flasks under the conditions indicated

Temperature of hot flask (°C)	*G. morsitans* Total No. of flies used	No. of flies attracted to hot flask	No. of flies attracted to cold flask	Percentage attracted to hot flask	*G. austeni* Total No. of flies used	No. of flies attracted to hot flask	No. of flies attracted to cold flask	Percentage attracted to hot flask
30	90	21	2	23	90	28	2	31
35	87	64	0	74	91	52	3	57
40	87	63	2	72	86	46	3	53
45	90	14	1	16	93	50	1	54
50	105	22	2	21	95	45	0	47
Totals	459	184	7		455	221	9	–

Table 2. Number of tsetse flies which fed or failed to feed on the sites indicated

Skin Area	Pelt thickness in mm	Mean fur length in mm	Radiant heat units	*G. morsitans* No. fed	No. unfed	% fed	*G. austensi* No. fed.	No. unfed	% fed
Rabbit									
Nostrils	2	3	20	–	–	–	3	2	60
Eyelids	–	–	24	8	6	57	5	0	100
Ears (inner)*	–	–	40	13	7	65	15	6	71
(outer)*	2	3	35						
Front foot	0–5	3	17	5	4	55	8	4	67
Hind foot	0–5	3	17	11	6	65	11	7	61
Anal region	–	–	20	–	–	–	3	2	60
Head	2–12	3–12	13	0	8	0	1	5	17
Neck	15–20	35	23	0	1	0	0	6	0
Thorax	10–15	30	17	0	5	0	0	2	0
Abdomen	10–15	35	16	0	21	0	0	31	0
Guinea-pig									
Nostrils	1.5	3	24	0	2	0	1	2	33
Eyelids	–	–	19	4	5	44	3	1	75
Ears (inner)	–	–	14	7	4	64	7	0	100
(outer)	1.5	7	14						
Front foot**	0–1	0–2	14	3	3	50	12	0	100
Hind foot**	0–2	0–2	11	7	3	70	15	13	53
Anal region	–	2	5	–	–	–	4	2	66
Head	0–5	3–15	5	1	8	11	0	2	0
Neck	15	15	9	–	–	–	0	1	0
Thorax	10–15	15	11	0	17	0	0	12	0
Abdomen	7–10	20	9	0	30	0	0	25	0

* Skin clearly visible between hair follicles; ** Bare skin between toes.

of 5.7 min, but only 5% fed on day 25 taking 14.2 min and no fly fed after day 25. These facts show that tsetse flies are inhibited from feeding where the pelt is more than 5 mm thick. Popham et al.[11] have shown that sounds emitted by gorged flies stimulate unfed flies to move and enable flies that have failed to feed to move to where blood may be more easily obtained. Unanaesthetized lizard, mice and gerbils attacked and killed 75 (61%) of 123 *G. austeni* placed in their cages - the agility of these small mammals preventing flies feeding on them. Guinea-pigs successfully killed 60 (78%) out of 77 specimens of *G. morsitans* and rabbits killed 17 (65%) out of 26 *G. austeni* used. These observations support others (unpublished) made in Kaduna, Nigeria in which wild lizards *(Agama agama)* attacked and destroyed *G. palpalis*. If reptiles and mammals behave similarly under natural conditions their absence from the records of Weitz[12] is understandable. These experiments show that although tsetse flies are attracted by radiant heat and to all parts of a host's skin, they only feed where the pelt is less than 1-5 mm thick.

1 Acknowledgments. Our gratitude is due to Dr A. Jordan of the Tsetse Fly Laboratories of the University of Bristol for supplying us with tsetse fly pupae, Mr J.A. Mynett of the Department of Mechanical Engineering of this University for his invaluable assistance in measuring energy flux and Dr W.R. Woof who has read and criticized the text.
2 C.F.M. Swynnerton, Trans. R. ent. Soc. Lond. *84*, 1 (1936).
3 F.L. Vanderplank, J. Anim. Ecol. *13*, 39 (1944).
4 J.Y. Moggridge, Proc. R. ent. Soc. Lond. A *23*, 86 (1948).
5 D.A. Turner, Trans. R. Soc. trop. Med. Hyg. *66*, 314 (1972).
6 D.A. Turner and J.F. Invest. Bull. ent. Res. *62*, 343 (1972).
7 J. Brady, Bull. ent. Res. *62*, 257 (1972).
8 G.A. Vale, Bull. ent. Res. *64*, 589 (1974).
9 J.A. Mynett, personal communication (1973).
10 T.A.M. Nash, Bull. ent. Res. *28*, 75 (1937).
11 E.J. Popham, M.J. Parr and V. Chowdhury, Experientia *34*, 1152 (1978).
12 B. Weitz, Bull. Wld Hlth Org. *25*, 1 (1962).

Heterotrophic growth in the blue-green alga *Anacystis nidulans*

S. Shanmugasundaram and M. Lakshmanan

Department of Microbiology, School of Biological Sciences, Kamaraj University (formerly Madurai University), Madurai 625021 (India), 10 August 1978

Summary. Anacystis nidulans was grown heterotrophically in the dark for the first time. Pyruvate served as the carbon source. The cells did not make pigments when grown heterotrophically, but synthesis of pigments resumed upon transfer of the cells to the light.

Blue-green algae were conventionally considered as obligate photoautotrophs. Many of the forms, including *Anacystis nidulans*, were however shown to take up organic compounds in light[1-6]. It was shown that filamentous forms such as *Anabaenopsis circularis*[7], *Anabaena cycadeae*[8], *Calothrix brevissima, C. membranica, Nostoc commune, Nostoc muscorum*[9], *Phormidium luridum, Plectonema boryanum*[9] and *Tolypothrix tenuis*[10] and the unicellular form *Aphanocapsa*[11] could grow in the dark. The unicellular form *Anacystis nidulans* and the filamentous form *Anabaena variabilis* were considered as obligate photoautotrophs, since they did not respond to external additions of carbon sources[12].

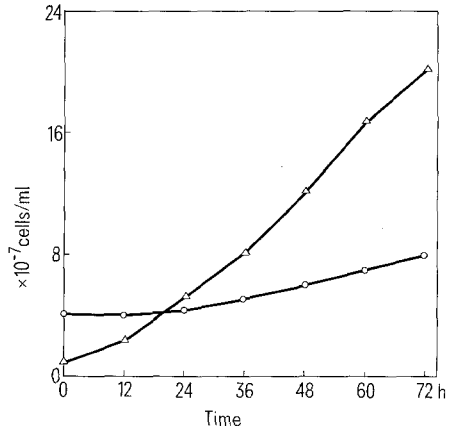

Fig. 1. Growth of *Anacystis nidulans* in autotrophic (△—△) medium in light (5000 lx) at 38 °C in a shaker and in heterotrophic medium in the dark at 32 °C as still culture (○—○).

Recently we have reported that dark-starved cells of *A. nidulans* could utilise pyruvate, α-ketoglutarate, succinate and glucose as respiratory substrates[13]. Employing plasmid-curing chemicals, such as acriflavin and acridine orange, we have isolated 'albino' cells of *A. nidulans* which were successfully cultivated at least for a few generations in an autotrophic medium supplemented with glucose[14]. The latter 2 experiments indicated the possibility of growing

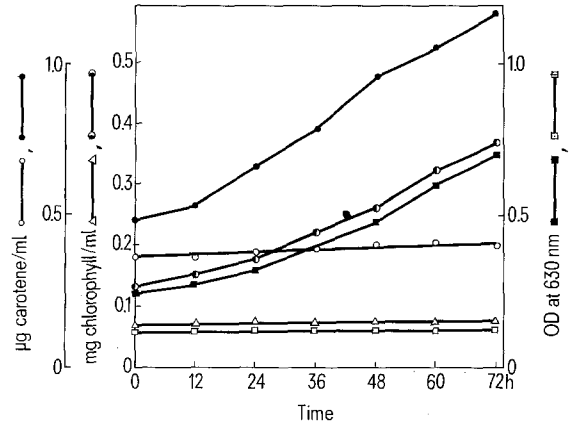

Fig. 2. Synthesis of pigments in light and dark grown cells. Chlorophyll (l ◐—◐, d △—△); carotenes (l ●—●, d ○—○); phycocyanin (l ■—■, d □—□); l, light grown cells; d, dark grown cells. Chlorophyll was extracted from the cells using 80% acetone and the pigment was estimated following the specific absorption coefficient of 82.0 at 663 nm. Phycocyanin was measured following the optical density of cells at 630 nm.