flowers[5,6], a search for a delphinidin derivative among the anthocyanins of roses was undertaken. In particular, blooms of well established purple and mauve varieties as well as those of the latest and bluest breeding lines were examined.

No delphinidin was found and a re-examination of the rose varieties reported to contain some myricetin[4] showed that only kampferol and quercetin were present. In the course of this survey, however, a third major anthocyanin was discovered in *Rosa rugosa* and derived varieties, e.g. 'Roseraie de L'Hay'. The pinkish red petals of these plants contain cyanin and the new pigment, which was readily identified as peonin (Tab. I). Since peonin is rare and has only previously been found in quantity in peony blooms[7], its presence in pink roses provides a valuable alternative source. Other new sources are the pink flowered garden geranium (Tab. I), a plant already known to contain pelargonin and malvin[2], and dark red varieties of *Lathyrus odoratus*[8].

Of the two previously known anthocyanins of roses cyanin is the most widely distributed, being present in all but two of the hundred or so varieties examined. The pelargonidin derivative, whose identity with pelargonin has now been confirmed, occurs in a number of scarlet varieties (e.g. 'Radar' and 'Will Scarlet') besides those already mentioned. Colour in the rose is therefore mainly due to pelargonin, cyanin or peonin or to mixtures of these pigments. Traces of the related 3-glucosides accompany these 3:5-diglucosides in some varieties. Purple or mauve colours are produced by co-pigmentation of cyanin; a fact which has been established by spectral measurements of aqueous acid extracts of the appropriate varieties (Tab. II).

Zusammenfassung. Im Gegensatz zu einer früheren Mitteilung konnten wir in den Blumenblättern der Hybriden-Tee-Rose kein Myricetin finden. Delphinidin kam in keiner der geprüften Rosenblüten vor. In roten Varianten erhält man violette Farben bei der «Copigmentation» von Cyanin. Pelargonin ist in gelbroten Rosen vorhanden und Peonin wurde erstmals in der rosa Variante gefunden.

Tab. I. Identification of peonin and pelargonin

Pigment and source		Spectral max in MeOH/HCl (in mμ)		Spectral ratio 440 mμ/max (as %)	Rf values in BAW	Rf values in BuHCl	Rf values in 1% HCl	Hydrolysis Products[a]
Peonin	authentic	274	523	13	0.31	0.10	0.17	peonidin, the 3- and 5-glucoside and glucose
	from *Rosa*	273	524	14	0.31	0.10	0.17	
	from *Pelargonium*	274	523	13	0.31	0.10	0.17	
Pelargonin	authentic	269	505	21	0.31	0.14	0.22	pelargonidin, the 3- and 5-glucoside and glucose
	from *Rosa*	266	507	20	0.31	0.14	0.23	

[a] For details of their identification see [6].

Tab. II. Co-pigmentation in mauve roses

Variety	Petal colour	Visual max in 1% aqu. HCl (in mμ)	Anthocyanidin formed on acid hydrolysis
'Belle Poitevine'	red	507	cyanidin
'Reine des Violettes'	violet	509	cyanidin
McGredy 56/944	violet blue	510	cyanidin
McGredy 55/1965	mauve	512	cyanidin

J. B. HARBORNE

John Innes Institute, Bayfordbury (Hertford, Herts), October 20, 1960.

[5] J. B. HARBORNE, Biochem. J. *68*, 12P (1958).
[6] J. B. HARBORNE, Biochem. J. *74*, 262 (1960).
[7] R. WILLSTÄTTER and T. J. NOLAN, Liebigs Ann. *408*, 136 (1915).
[8] J. B. HARBORNE, Nature, Lond. *187*, 240 (1960).

Rate of Respiration in Relation to Autogamy in *Paramecium aurelia*

It is well known that cultures of paramecia in which autogamy (self-fertilisation) and conjugation (cross-fertilisation) are prevented by daily re-isolation into fresh medium undergo a progressive process of aging. Rejuvenescence can be obtained by allowing autogamy (or conjugation) to take place. A point is finally reached, however, when rejuvenescence can no longer be obtained by this procedure and the clone is doomed to extinction[1]. The theoretical basis of this phenomenon is as yet obscure—rejuvenescence is dependent on a reproductive process involving reconstitution of the nuclear apparatus of the organism by a meiotic process (autogamy or conjugation) in which there is no segregation of genetic material since homozygous lines can be used with equal facility.

In the present investigation measurements have been made of the rate of respiration of single paramecia from serial isolation cultures before and after the occurence of autogamy in order to elucidate the nature of the metabolic changes occuring as a consequence of autogamy.

The stocks of paramecia used were selected at random and belong to two syngens (varieties) of the species *Paramecium aurelia*; namely stock 60, syngen 1 (collected originally in Burlington, Virginia), and stock 39, syngen 9 (isolated originally from the river Eure, France).

Respiration was measured by Cartesian diver microrespirometry and the apparatus and methods employed were similar to those developed by LINDERSTRØM-LANG[2,3] and HOLTER[4] with only minor modification. In order to facilitate comparison two divers only were used throughout. A single paramecium of known physiological status was loaded into the diver under sterile conditions. The charge containing the paramecium consisted of standard culture medium (an infusion of dried lettuce innoculated with a strain of *Aerobacter aerogenes*) passed through a

[1] T. M. SONNEBORN, J. Protozool. *1*, 38 (1954).
[2] K. LINDERSTRØM-LANG, Nature, Lond. *140*, 108 (1937).
[3] K. LINDERSTRØM-LANG, C. R. Lab. Carlsberg, Sér. Chim. *24*, 333 (1943).
[4] H. HOLTER, C. R. Lab. Carlsberg, Sér. Chim. *24*, 399 (1943).

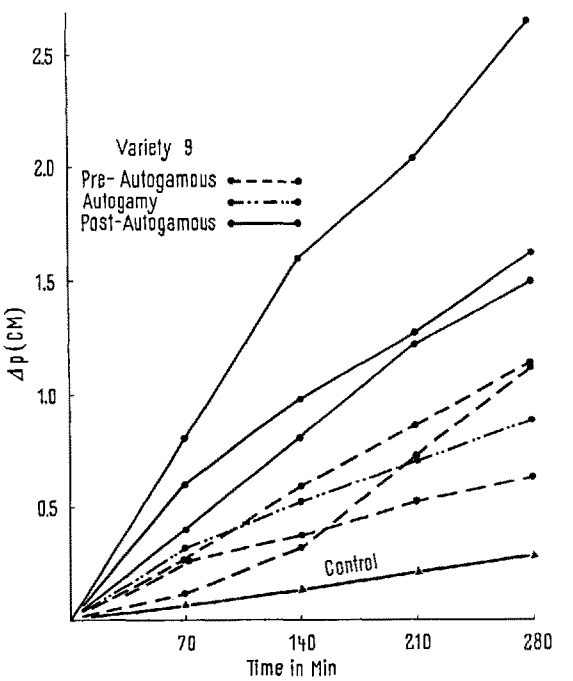
Variety	Stock	Stage	Duration of Experiment (min)	Diver	Mean Δp/h less control value (cm)	Mean oxygen consumption (µl/h)	
1	60	Pre-autogamous	280	D_1	0.098	1.531	1.227
1	60	Pre-autogamous	280	D_1	0.059	0.923	
1	60	Post-autogamous	280	D_1	0.265	4.150	4.100
1	60	Post-autogamous	280	D_1	0.259	4.050	
9	39	Pre-autogamous	280	D_2	0.168	2.535	
9	39	Pre-autogamous	280	D_2	0.173	2.599	
9	39	Pre-autogamous	280	D_2	0.063	0.955	1.823
9	39	Pre-autogamous	280	D_1	0.104	1.623	
9	39	Pre-autogamous	280	D_1	0.093	1.460	
9	39	During autogamy	280	D_2	0.117	1.762	1.544
9	39	During autogamy	280	D_1	0.085	1.326	
9	39	Post-autogamous	280	D_2	0.273	4.008	
9	39	Post-autogamous	280	D_2	0.497	7.480	
9	39	Post-autogamous	280	D_2	0.250	3.753	5.506
9	39	Post-autogamous	280	D_1	0.183	2.879	
9	39	Post-autogamous	280	D_1	0.248	3.880	
Mean Δp/h for control divers (i.e. no paramecium in diver)			480	$D_1 + D_2$	0.072		

Hemmings filter to remove the bacteria. The paramecium was freed of bacteria by repeated washings in sterile culture fluid. The respiration measurements were carried out at 26°C, which was also the temperature of incubation of the parent cultures. The pre-autogamous animals were taken from a homozygous clone which had undergone 50–150 divisions in the absence of autogamy. The post-autogamous animals were derived from the same cultures and the respiration measurements were carried out 2–3 divisions after autogamy. The procedure for controlling the occurence of autogamy in laboratory cultures has been described by SONNEBORN[5].

The Figure illustrates the results obtained with one of the divers. The respiration measurements with each diver must be plotted separately since the gradients of the lines (Δp) is a function of the diver constant. The calculated values for the rates of oxygen consumption (in µl/h) are given in the Table. These results demonstrate the feasibility of measuring the respiratory rate of single paramecia at specific phases of their life cycle. So far they indicate that there is a marked increase in oxygen consumption after autogamy in comparison with the pre-autogamous rate. The difference is statistically significant in the case of the syngen 9 figures (t test; $p < 0.001$). There is no indication in the figures at present available of an inter-stock difference of comparable magnitude. The rate during autogamy (at anlagen formation) appears to be similar to the pre-autogamous rate.

The values for the rates of oxygen consumption are of the same order as those obtained by CUNNINGHAM and KIRK[6] for *P. caudatum*, but somewhat larger than those obtained by some other workers[7–10]. It has been shown, however, that culture conditions (density) markedly affect oxygen consumption[11]. Consequently higher values would be expected in this case since the measurements were made on single animals and not on mass cultures.

In the case of plant and animal tissues it has been shown that a high respiration rate is associated with youth and that there is a progressive decline in the rate of oxygen consumption of tissues with age[12–16]. It would appear, therefore, that the aging phenomenon observed in paramecia cultures is analogous to the aging of plant and animal tissues.



Respiration rates of single paramecia of Variety 9 before, during and after autogamy (Diver D_2).

[5] T. M. SONNEBORN, J. exp. Zool. *113*, 87 (1950).
[6] B. CUNNINGHAM and P. L. KIRK, J. cell. comp. Physiol. *20*, 119 (1942).
[7] J. O. W. BARRATT, Z. allg. Physiol. *5*, 66 (1905).
[8] R. B. HOWLAND and A. BERNSTEIN, J. gen. Physiol. *14*, 339 (1931).
[9] B. A. HUMPHREY and G. F. HUMPHREY, J. exp. Biol. *25*, 123 (1948).
[10] D. H. SIMONSEN and W. J. VAN WAGTENDONK, Biochim. biophys. Acta *9*, 515 (1952).
[11] D. M. PACE and K. K. KIMURA, J. cell. comp. Physiol. *24*, 173 (1944).
[12] O. ROSENTHAL, M. A. BOWIE, and G. WAGONER, J. cell. comp. Physiol. *17*, 221 (1941).
[13] H. A. RAFSKY, B. NEWMAN, and A. HORONICK, J. Geront. *7*, 38 (1952).
[14] M. D. ALLEN, J. exp. Biol. *36*, 92 (1959).
[15] W. A. HIMWICH, H. B. W. BENARON, B. E. TUCKER, C. BABUNA, and M. C. STRIPE, J. appl. Physiol. *14*, 873 (1959).
[16] C. MUIR, L. L. DEKOCK, P. C. DEKOCK, and R. H. E. INKSON, Exper. *15*, 354 (1959).

Zusammenfassung. Die Atmung einzelner Pantoffeltierchen (*Paramecium aurelia*) aus Serien-isolierten Kulturen wurde mit Hilfe eines Cartesischen Tauchers gemessen. Die Messungen an Individuen vor und nach Autogamie (Selbstbefruchtung) zeigten, dass die Atmung nach Autogamie in signifikanter Weise ansteigt.

Da die Autogamie für das Überleben der Kulturen eine grosse Wichtigkeit besitzt, ergeben sich Parallelen zwischen den Stoffwechselveränderungen bei *Paramecium* und der Alterung der somatischen Zellen von Metazoen.

C. R. PRINGLE and J. M. STEWART

Zoology Department, University of Aberdeen (Scotland), November 5, 1960.

Vitamin Requirements of *Nemalion multifidum*

In axenic culture, the red alga *Goniotrichum elegans* was found to be a vitamin heterotroph[1,2]. Like many other marine organisms, it required cyanocobalamin for growth. In November 1959, success was achieved in growing another red alga in axenic culture, *Nemalion multifidum* (WEBER and MOHR) J. Ag. a member of *Florideae*. The method for sterilization will be described in a later paper. The method for cultivation was the same as that earlier used by the author[2]. The alga was grown in the artificial medium ASP 6[3] with nitrate replaced by asparagine. During the first months, the alga seemed to be autotrophic. Cultures in a vitamin-containing substrate grew as rapidly as those in a vitamin-free solution. Subsequently, however, the vitamin-starved cultures became pale in colour, and additions of the vitamin solution of ASP 6, a mixture of 15 different vitamins, increased growth up to 100%. Eleven of the vitamins were sterilized together in an autoclave ('autoclaved vitamins') but the remainder, pyridoxamine, lactoflavin, folic acid and cyanocobalamin, were passed through a sterile glass filter before being added to the sterile flasks. The active substance could be located as being among these four vitamins (Tab. I). Cyanocobalamin (= B_{12}) had the strongest effect, but pyridoxamine also caused an increase in growth.

In order to determine which part of the cyanocobalamin molecule was necessary, different B_{12}-analogues were added (Tab. II). At that time, the inocula had been starved for four months and even additions of cyanocobalamin increased growth very little. However, Factor 1b (= Factor B ribosephosphate) was the more active one; while Factor B, which contains only the pseudophorphyrine ring, had no effect. *Nemalion* was very little affected by such analogues as Factor III or Factor Z1, in contrast to the behaviour of *Goniotrichum elegans*.

In the early experiments, not only pyridoxamine but also pyridoxine increased the growth of Nemalion. When added together with B_{12} or Factor 1b, pyridoxamine gave a higher yield than these compounds alone. When the inocula became more starved this effect did not appear (Tab. III).

In all these experiments, some growth occurred also in the controls; and thus a very weak vitamin production is indicated. An addition of one vitamin can thus enhance growth while there is some production of other necessary compounds but not sufficient for optimal growth. The cyanocobalamin molecule seems to be broken down before its incorporation in an enzyme and the Factor 1b molecule without the benzimidazole ring is more conveniently utilized for this purpose. The role which the B_6 vitamins play in this connection cannot yet be explained, but a cooperation between B_{12} and B_6 is known from the methionine synthesis of a B_6-less mutant of *Escherichia coli*[4].

Tab. I. Growth of *Nemalion multifidum* with different vitamins

Substance added	Dry weight in mg of algae from 6 flasks	
	Experiment 1 Incubation time 24 days	Experiment 2 Incubation time 20 days
0	6.4	5.8
All vitamins	10.6	—
'Autoclaved vitamins'	8.6	—
Pyridoxamine, lactoflavin, folic acid, B_{12}	13.8	9.7
Pyridoxamine	12.8	8.9
Lactoflavin	5.8	—
Folic acid	7.4	7.2
B_{12}	14.2	9.9
B_{12} + pyridoxamine	—	10.8

Tab. II. Growth of *Nemalion multifidum* with different B_{12}-analogues

Additions of B_{12}-analogues 1 µg/l	Dry weight in mg of algae from 6 flasks
0	12.7
B_{12}	14.0
Factor B	13.0
Factor III	14.0
Pseudovitamin B_{12}	13.8
Factor 1b	32.9
Factor Z1	14.3
Factor Z2	14.4
Incubation time 42 days.	

Tab. III. Growth of *Nemalion* with additions of B_{12} or Factor 1b together with pyridoxamine and B_6

Addition	Dry weight in mg of algae from 6 flasks		
	Exper. 1	Exper. 2	Exper. 3
0	4.2	4.8	2.6
B_{12}	4.8	—	—
Factor 1b	17.0	11.1	9.0
B_6	4.0	—	—
Pyridoxamine	4.2	5.6	—
B_{12} + B_6	5.0	—	—
B_{12} + pyridoxamine	5.8	—	—
Factor 1b + B_6	21.4	—	—
Factor 1b + pyridoxamine	23.2	10.4	7.4
Incubation time 30 days.			

[1] L. FRIES, Nature *183*, 558 (1959).
[2] L. FRIES, Physiol. Plantar. *13*, 264 (1960).
[3] L. PROVASOLI, J. J. MCLAUGHLIN, and M. R. DROOP, Arch. Mikrobiol. *25*, 392 (1957).
[4] S. WIJESUNDERA, M. J. CROSS, and D. D. J. WOODS, Gen. Microbiol. *22*, 786 (1960).