freed from underlying mesoderm and then cut at random into 1/2, 1/4, and 1/6 parts. A similar square piece from the other side (Fig. B) was left intact as control. Sterile Holtfreter buffered (after DEUCHAR[10]) was used throughout the experiments and this contained a mixture of 15,000 i.u./l of Penicillin and Streptomycin. These small isolates, together with the controls, were cultured for 48 h. During this period, some of the isolates lost quite a number of cells and were rejected. The healthier ones were wrapped up either with *Triturus* or with *Xenopus* ectoderm and cultured for another 72 h. *Xenopus* ectoderm was mostly used in these experiments. The materials were fixed in Bouins. Sections were cut at 10 μ and stained either with celestine blue or with celestine blue and eosin.

Results and discussion. The amount of differentiation of the isolates varied from neural palisade to neural tube. This is shown in the following Table.

Size of the isolate	Total number of cases	Number of cases with differentiation	% of different pieces
1	7	7	100·0
1/2	21	18	86·0
1/4	31	22	71·0
1/6	25	17	68·0

It is clear from the small series of experiments that neural differentiation gradually dropped with the decrease in the size of the isolate down to the limiting value of 1/6. This size allowed the isolate to remain viable and undergo differentiation. Fragmentation beyond 1/6 was rather difficult as nearly all the fragments disintegrated soon after they were made.

GROBSTEIN[7] observed that with the mouse and chick materials 1/8 was the 'critical mass' and 1/16 part never achieved this when left alone, but underwent neural differentiation when combined in 'close cluster'.

It seems from the present experiments that, in *T. alpestris* material, 1/6 is the 'critical mass' to undergo neural differentiation.

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Résumé

On a détaché de l'ectoderme présomptif neural de migastrulae de *Triturus alpestris* des lambeaux de grandeur définie qui ont été sectionnés au hasard en fragments d'une demi, d'un quart et d'un sixième, puis emballés dans de l'ectoderme de *Xenopus* ou *Triturus* et mises en culture dans la solution d'Holtfreter. Il a été constaté que le % des cas où une différenciation neurale s'est produite décroît avec la taille du fragment.

[10] E. DEUCHAR, J. exp. Biol. *30*, 18 (1953).

The Effect of Age on the Responses of Animal and Plant Tissues to Metabolic Inhibitors

An observation that the respiratory responses of tissues to metabolic inhibitors showed similar changes with age in plants and animals led to a series of experiments summarised below.

(i) Respiration rates of slices from the brains of 1–3-day and 14–17-month old rats were determined in the presence and absence of different metabolic inhibitors (Table I and II). The rats were killed by a blow on the neck, the brain dissected out, halved, and weighed. Each half was cut into thin slices and placed in one of a pair of Warburg vessels containing Krebs-Ringer solution[1] plus 2% glucose, and one of which contained in addition the dissolved inhibitor. Oxygen uptakes were determined at 37·2°C by conventional methods[1]. Studies using cyanide were carried out according to ROBBIE[2]. Two sets of experiments were performed, one in 1956 using 14-month old rats (Table I) and one in 1958 using 17-month old rats (Table II).

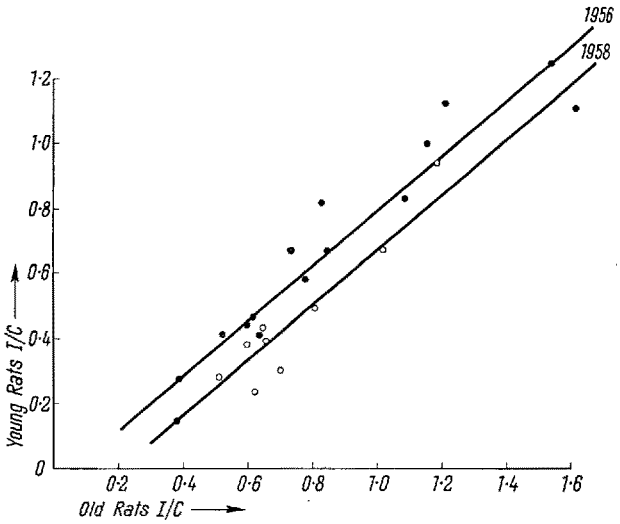

Figure 1. – The ratio (I/C) of respiration rate of young and old rat brain in presence (I) or in absence (C) or various respiratory inhibitors.

A statistical analysis of the differences in oxygen uptake for control (C) and inhibited (I) units was considered. The variability of this difference over the set of inhibitors and concentrations used, was greater for young rats than for old rats. Consequently separate within-animal standard errors, based on 23 degrees of freedom, are presented for testing the overall differences ($C - I$). The mean values for all 24 trials are presented in Table III. The presence of inhibitors reduces markedly the oxygen uptake of young rat brain tissue. This contrasts with the much smaller and non-significant effect of inhibitors on old brain, where the mean oxygen uptake is much lower than that of young rat brains.

When the ratio of 'inhibitors' to 'control' respiration rates (I/C) was calculated for each concentration of each inhibitor for old rats (denoted by x) and for young rats (denoted by y) and regression lines of the form $y = a + bx$ were fitted for the 1956 and 1958 sets of data (Fig. 1), it was found that a pooled analysis could

[1] W. W. UMBREIT, R. H. BURRIS, and J. F. STAUFFER, *Manometric Techniques* (Burgess Publish. Co., Minneapolis 1957).

[2] W. A. ROBBIE, *Methods in Medical Research* (Edit. V. R. POTTER, Year Book Pub., Chicago 1948).

Table I
The effect of various inhibitors on the respiration of old and young rat brains compared. Experiments of 1956

Inhibitor	Molar Concentration		Oxygen µl/g fresh weight/h		*I/C*	
			Old	Young	Old	Young
Cyanide	5×10^{-4}	*C*	104	324	0·78	0·58
		I	81	189		
	5×10^{-4}	*C*	291	458	0·38	0·14
		I	111	64		
	10^{-4}	*C*	231	824	1·62	1·11
		I	375	913		
	10^{-4}	*C*	399	959	1·54	1·25
		I	614	1198		
Azide	10^{-3}	*C*	233	440	0·83	0·82
		I	194	360		
	10^{-3}	*C*	238	470	0·85	0·67
		I	202	316		
	5×10^{-4}	*C*	171	223	0·64	0·41,
		I	110	92		
Malonate.	2×10^{-2}	*C*	189	423	0·39	0·27
		I	73	115		
o-Phenanthroline 2:4	10^{-3}	*C*	436	482	0·52	0·41
		I	225	197		
DNP	10^{-3}	*C*	223	438	0·62	0·46
		I	138	201		
	10^{-4}	*C*	310	518	1·09	0·83
		I	338	429		
	$3 \cdot 3 \times 10^{-5}$	*C*	247	151	1·21	1·12
		I	298	169		
	2×10^{-5}	*C*	282	461	1·16	1·00
		I	327	461		
Diethyldithiocarbamate	2×10^{-3}	*C*	281	613	0·74	0·67
		I	209	413		
Iodoacetate	2×10^{-3}	*C*	294	526	0·60	0·44
		I	175	231		

Table II
The effect of various inhibitors on the respiration of old and young rat brain compared. Experiments of 1958

Inhibitor	Molar Concentration		Oxygen µl/g fresh weight/h		*I/C*	
			Old	Young	Old	Young
Cyanide	2×10^{-4}	*C*	230	634	0·65	0·43
		I	149	274		
	10^{-4}	*C*	191	633	0·81	0·49
		I	154	313		
Azide	2×10^{-4}	*C*	191	386	0·51	0·28
		I	98	109		
Malonate.	10^{-2}	*C*	215	653	0·70	0·30
		I	149	193		
o-Phenanthroline . . .	10^{-3}	*C*	152	682	0·66	0·39
		I	101	268		
2:4 DNP	2×10^{-4}	*C*	216	572	0·60	0·38
		I	131	217		
Diethyldithiocarbamate	2×10^{-3}	*C*	207	687	1·19	0·94
		I	246	642		
Iodoacetate	2×10^{-3}	*C*	266	471	0·62	0·24
		I	165	111		
Na Fluoride	10^{-3}	*C*	317	743	1·02	0·67
		I	322	499		

be carried out on the two sets of data and further that the slopes of the two lines did not differ significantly. The distance between these parallel lines was then tested and found to differ significantly from zero. Thus the situation reduced to having two parallel lines of the same slope, the equations for which are:

$$y = 0{\cdot}853\,x - 0{\cdot}059 \text{ for 1956;}$$
and $$y = 0{\cdot}853\,x - 0{\cdot}183 \text{ for 1958.}$$

The standard error of the regression coefficient, 0·853, is $\pm$ 0·0595.

It was found that when such brains were homogenised,

Table III
Mean oxygen uptake of old and young rats in presence (*I*) and absence (*C*) of various inhibitors (µl/g fresh weight/h)

	C	*I*	*C-I*
Old rats	246	208	38 ± 19·2
Young rats	532	332	200 ± 35·7

the differences in respiration rate and response to inhibitors largely disappeared, a result in accord with those of WEINBACH and GARBUS[3] and of REINER[4] but in contrast to these workers, a 'physiological' salt solution was used. The homogenizing would however still produce an unnatural environment in which the enzymes were active.

(ii) Since the recorded oxygen uptake in the experiments were somewhat lower than those given by KREBS[5], testis tissue was chosen because the seminal tubules would easily separate from one another, and the thickest tubules present would not exceed the 0·3 mm dimensions critical for oxygen diffusion[1].

Four concentrations of cyanide, azide, and 2:4 dinitrophenol were used. The oxygen uptakes and *I/C* ratios are presented in Table IV. The data allowed a test of the difference between the within- and between-inhibitors regression. These two regressions do not differ significantly and thus the degree of association between the *I/C* ratios for young and old material is unaffected by the inhibitor factor. It was found that the situation could be adequately represented by the total regression equation:

$$y = 0{\cdot}051 + 0{\cdot}793\,x\ (S.E. \pm 0{\cdot}1023).$$

It could be concluded therefore that the difference between old and young brains could not be attributed entirely to thickness of slice nor differences in permeability to various inhibitors.

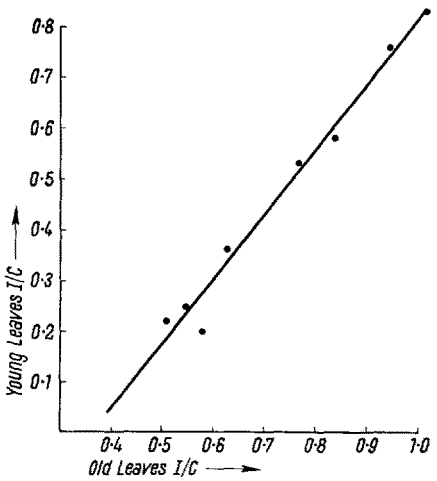

Figure 2.—The ratio (*I/C*) of respiration rate of young and old tomato leaves in presence (*I*) and in absence (*C*) of various respiratory inhibitors.

(iii) Similar studies were carried out using the uppermost fully expanded leaf and the 6th leaf of a series of uniform tomato plants, grown in water culture in a greenhouse[6]. The *I/C* ratios obtained from these leaves are

[3] E. C. WEINBACH and J. GARBUS, Nature *178*, 1225 (1956).
[4] J. M. REINER, J. Gerontol. *2*, 315 (1955).
[5] H. A. KREBS, Biochim. Biophys. Acta *4*, 249 (1950).
[6] I. R. MACDONALD and P. C. DEKOCK, Physiol. Plant. *11*, 464 (1958).

Table IV
The oxygen uptake of old and young rat testis in presence (*I*) or absence (*C*) of various concentrations of cyanide, azide, or 2:4 dinitrophenol (DNP)

Inhibitor	Molar Concentration		Oxygen uptake µl/g fresh weight/h		*I/C*	
			Old	Young	Old	Young
Cyanide	2×10^{-4}	*C*	415	430	0·52	0·55
		I	215	236		
	2×10^{-4}	*C*	400	415	0·28	0·22
		I	112	93		
	1×10^{-4}	*C*	400	415	0·35	0·33
		I	140	136		
	1×10^{-5}	*C*	309	415	1·04	0·86
		I	319	355		
Azide	2×10^{-4}	*C*	364	510	0·45	0·52
		I	165	265		
	2×10^{-4}	*C*	375	490	0·53	0·47
		I	200	230		
	1×10^{-3}	*C*	375	490	0·43	0·36
		I	160	175		
	1×10^{-4}	*C*	375	490	0·60	0·53
		I	225	260		
DNP	2×10^{-4}	*C*	276	516	0·57	0·41
		I	158	213		
	2×10^{-4}	*C*	330	357	0·36	0·25
		I	120	90		
	1×10^{-4}	*C*	330	357	0·38	0·41
		I	125	145		
	5×10^{-5}	*C*	330	357	0·48	0·45
		I	160	162		

Table V
The effect of varying concentrations of different inhibitors on young and old tomato leaf respiration

Inhibitor	Molar Concentration		Oxygen uptake µl/100 mg dry weight/h		I/C	
			Old	Young	Old	Young
Cyanide	5×10^{-4}	C	2·12	3·13	0·58	0·20
		I	1·23	0·63		
	25×10^{-4}	C	1·96	2·90	0·51	0·22
		I	1·00	0·63		
DNP	1×10^{-4}	C	1·52	3·01	0·84	0·58
		I	1·27	1·74		
	1×10^{-3}	C	1·83	2·87	0·55	0·25
		I	1·01	0·72		
$CO:O_2$ (80:20)	–	C	1·64	2·90	1·02	0·83
		I	1·68	2·40		
	–	C	1·80	2·91	0·77	0·53
		I	1·38	1·53		
o-Phenanthroline	1×10^{-3}	C	1·58	3·00	0·95	0·76
		I	1·50	2·29		
Sodium Azide	1×10^{-3}	C	1·59	2·78	0·63	0·36
		I	1·00	1·01		

recorded in Table V. The regression equation for these points is: $y = 1{\cdot}267\,x - 0{\cdot}460$ as shown in Figure 2. The standard error of this regression coefficient is $\pm$ 0·0742.

The significance of the proportional responses of old and young leaves has already been discussed[6–8]. In these papers it is suggested[7] that the ratio of total Phosphorus to Iron content and Potassium to Calcium in the tissues gives some indication of the metabolic state of the plant cell. In [6] the relationship between Phosphorus content and available Iron and the effect of this Iron on the metabolism of the cell is discussed and also the effect of respiration inhibitors.

It is evident that similar trends are obtained for both animal and plant tissues and that the following generalisations would be valid for both groups:

The proportionality of response of old and young tissues to inhibitors, irrespective of their nature, site of action or concentration, indicates

(*a*) that the metabolism of the cell is a regulated entity and not a series of separate processes each of which has its own limiting factor,

(*b*) that the balance between the various processes changes with ageing of the organism and to some extent can be used to reflect the age of the organism,

(*c*) that no metabolic system is lost or new system introduced during the life of the organism.

Supporting evidence for the above views can be found in published analyses of animal tissues (Lowry and Hastings[9], Lansing[10], Gans[11], Pearson[12]).

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[7] P. C. deKock and A. Hall, Plant Physiol. *30*, 293 (1955).
[8] P. C. deKock and R. I. Morrison, Biochem. J. *70*, 272 (1958).
[9] O. H. Lowry and A. B. Hastings, *Cowdray's Problems of Ageing* (The Williams & Wilkins Comp., Baltimore 1952).
[10] A. I. Lansing, Biol. Bull. *82*, 392 (1942).
[11] H. Gans, Brain *46*, 178 (1923).
[12] P. B. Pearson, J. biol. Chem. *106*, 1 (1934).

Zusammenfassung

Junge Gewebe von Ratten zeigen eine grössere Empfindlichkeit gegenüber verschiedenen Konzentrationen von Atmungsinhibitoren als alte Gewebe. Ähnliche Resultate wurden bei der Einwirkung derselben Inhibitoren auf junge und alte Blätter von Tomatenpflanzen erzielt. Die Unterschiede sind statistisch signifikant. Es wird angenommen, dass keine neuen metabolischen Systeme während des Lebens eines Organismus gebildet werden, sondern dass während des Alterns nur eine Verschiebung im Gleichgewicht dieser Systeme eintritt.

PRO EXPERIMENTIS

A Simplified Method of Preparing Buffered Egg-Yolk Extracts Used as Diluter for Human and Bull Semen

Developing a method for the study of the kinetics of the killing of spermatozoa, we came across the need for an optically empty diluter which does not in any way impair the motility or the chance of survival of the spermatozoa. Considering the very advantageous properties of egg-yolk diluters, we set out with the idea of preparing a buffered extract of egg-yolk with the above-mentioned properties. Without knowledge of the work being done in this field by Rikmenspoel[1], we applied similar methods, i.e. ultracentrifugation and repeated ultrafiltration. Although the result was satisfying, this mode of preparing the diluter appeared too laborious and expensive to permit its extensive use. However, during this work it was observed that a suspension of egg-yolk in citrate buffer settles down spontaneously, giving a slightly opaque supernatant.

Mode of preparation. One volume of egg-yolk, thoroughly freed from egg-white and yolk membrane, is stirred for 5 min with 2 volumes of citrate buffer (*M* 0·986, pH 7·4)

[1] R. Rikmenspoel, Exper. *13*, 124 (1957).