

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

Inhibitor	Molar Concentration	Oxygen uptake $\mu\text{l}/100 \text{ mg dry weight/h}$		I/C		
		Old	Young	Old	Young	
Cyanide . . . . .	$5 \times 10^{-4}$	C	2.12	3.13	0.58	0.20
		I	1.23	0.63		
	$25 \times 10^{-4}$	C	1.96	2.90	0.51	0.22
		I	1.00	0.63		
DNP . . . . .	$1 \times 10^{-4}$	C	1.52	3.01	0.84	0.58
		I	1.27	1.74		
	$1 \times 10^{-3}$	C	1.83	2.87	0.55	0.25
		I	1.01	0.72		
CO:O <sub>2</sub> (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 . . . .	$1 \times 10^{-3}$	C	1.58	3.00	0.95	0.76
		I	1.50	2.29		
Sodium Azide . . . . .	$1 \times 10^{-3}$	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:  $y = 1.267x - 0.460$  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<sup>6-8</sup>. In these papers it is suggested<sup>7</sup> 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<sup>8</sup> 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<sup>9</sup>, LANSING<sup>10</sup>, GANS<sup>11</sup>, PEARSON<sup>12</sup>).

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<sup>7</sup> P. C. DEKOCK and A. HALL, *Plant Physiol.* 30, 293 (1955).

<sup>8</sup> P. C. DEKOCK and R. I. MORRISON, *Biochem. J.* 70, 272 (1958).

<sup>9</sup> O. H. LOWRY and A. B. HASTINGS, *Cowdrey's Problems of Ageing* (The Williams & Wilkins Comp., Baltimore 1952).

<sup>10</sup> A. I. LANSING, *Biol. Bull.* 82, 392 (1942).

<sup>11</sup> H. GANS, *Brain* 46, 178 (1923).

<sup>12</sup> 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<sup>1</sup>, 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)

<sup>1</sup> R. RIKMENSPOEL, *Exper.* 13, 124 (1957).

Table I

Amount of nitrogen and phosphorus in different samples of diluter

Sample No.	mg N/ml	mg P/ml
1	7.37	1.67
2	7.36	1.66
3	7.36	1.70
4	7.34	1.69

being 0.015 *M* as to sulphanilamide, and containing 335 IU of benzylpenicilline (sodium salt, Glaxo) per ml. After thorough mixing, the suspension is left at + 2.0°C for 48 h, after which time the supernatant is pipetted off.

*Properties.* The liquid obtained shows a yellowish colour and a feeble turbidity when inspected in a thin layer. Yolk particles of a size comparable with that of the sperm heads are very scarce. Smaller particles with a diameter below 1  $\mu$  are abundant, but do not consistently disturb the dark background at darkfield illumination. The pH varies between 6.90 and 7.00. Analyses of the content of N and P show small variations from sample to sample (Table I). This diluter may be stored at + 2°C for weeks without losing its properties.

Table II

Percentage of mobile bull spermatozoa kept at + 4°C in diluter

Samples No.	1		2		3	
	4	24	4	24	4	24
Spermatozoa giving 'scatting tracks' <sup>2</sup> . . . . .	56	37	65	55		
Spermatozoa progressing without rotation . . . . .	23	32	23	25		
Spermatozoa swimming in circles . . . . .	1	2	—	—		
Movement unclassified . . . . .	1	2	1	1		
Total mobile . . . . .	81	73	89	81	81	79

So far we are only able to judge the effect of this diluter on the survival of spermatozoa from observations on mobility.

Such observations on second ejaculates from three different bulls are presented in Table II. The semen was diluted 1:100 immediately after being collected, and the mobility registered after 4 and 24 h using a special photographic method which will be described elsewhere. In a similar experiment with human spermatozoa, the semen was diluted ten times only after 4 h, the semen being kept at room temperature before dilution. During the following 20 h at + 4°C the percentage of the mobile spermatozoa decreased from 81 to 70.

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#### Zusammenfassung

Es wird eine vereinfachte Methode zur Herstellung eines isotonischen zittrathaltigen Extraktes von Eidotter

beschrieben, der frei von Dotterteilchen grösser als 1  $\mu$  ist. Die Konstanz der Zusammensetzung bei wiederholter Herstellung dieses Extraktes sowie sein Einfluss auf die Motilität von Spermatozoen werden untersucht.

#### PRO EXPERIMENTIS

#### A Simple Objective Method for Determination of the Glare Effect

(Preliminary report)

On the whole, there are two ways in which glare could affect the visual ability. One is caused by a lowered foveal sensitivity when a strong light falls into the eye (adaptive or retinal type). The other type appears when the eye is exposed to a light falling from the side. In this case, there is a diffuse diffraction and scattering of light in the optic media throwing an extra veil of light on the image of the object whereby the brightness of the object as well as of its surroundings is heightened (diffuse or veiling type). (For a survey of the physiology of glare, see e.g. GOLDMANN<sup>1</sup>.)

The effect of glare on vision has generally been determined by subjective methods, based on the report of a test person. A more objective method of determining the visual ability is to make use of optokinetic nystagmus, registered electro-nystagmographically. In view of the fact that investigations of this type have not been carried out previously for the purpose of studying the glare effect, this preliminary report has been made with the object of drawing attention to a new and simple method which, in addition, happens to be as objective as could reasonably be expected.

*Method.* Optokinetic nystagmus was produced by projecting on to a screen, in the shape of a semi-circular cylinder, a number of vertical black and white stripes of equal width which were caused to move horizontally across the screen. The illumination was 20 lux. A detailed description of the apparatus will be found in a paper submitted by BLOMBERG<sup>2</sup>. The eye movements in the horizontal plane were registered electro-nystagmographically in the customary manner. Use was also made of vertical electrodes to note any possibly occurring blinks in the course of the experiment. The movement of the stripes was adjusted at optimum speed, i. e. the speed at which maximum frequency and highest amplitude of optokinetic nystagmus were achieved. The experiments took place in a darkened room.

In order to produce glare of the adaptive type, a photographic flash (duration 1/1000 s) was triggered behind the individual undergoing the experiment. The flash was directed towards the ceiling. For the purpose of preventing the flash from causing consternation, with eye-shutting or blinking as a natural consequence, the test subject was told beforehand of the sequence of procedure. Attempts were also made to bring about diffuse glare by transmitting a beam of light from an ordinary torch held at a distance of about 20 cm from the eye in the orbital plane, from directly ahead, and from the side, at an angle of about 45 degrees to the visual axis. The other eye was held shut during this experiment.

*Results.*—It was found that the glare caused by the flashlight exposure had the effect of abolishing optokinetic

<sup>1</sup> E. GOLDMANN, Bull. Schweiz. elektrotechn. Ver. 41, 751 (1950).

<sup>2</sup> L. ROTSCHILD, *Mammalian Germ Cells*, CIBA Found. Symp. (London 1953), p. 122.

<sup>2</sup> L.-H. BLOMBERG, *The 'optokinetic fusion limit'. A study in 55 healthy persons.* (To be published.)