p-Bromobenzyl chloroformate was then prepared from *p*-bromobenzyl alcohol, as a stable low-melting crystalline solid. With glycine it gave carbo-*p*-bromobenzyloxyglycine (m.p. 139°) (found: C, 41.8; H, 3.3. $C_{10}H_{10}O_4NBr$ requires: C, 41.7; H, 3.5 per cent); treatment of the latter with phosphorus pentachloride followed by coupling with glycine ethyl ester gave carbo-*p*-bromobenzyloxyglycylglycine ethyl ester (m.p. 125°) in nearly theoretical yield (found: C, 45.3; H, 4.5. $C_{14}H_{17}O_5N_2Br$ requires: C, 45.04; H, 4.56 per cent). With hydrazine, carbo-*p*-bromobenzyloxyglycylglycyl hydrazide was obtained (m.p. 180°) (found: C, 40.5; H, 4.3. $C_{13}H_{16}O_4N_4Br$ requires: C, 40.12; H, 4.18 per cent). With glycine ethyl ester, *p*-bromobenzyl chloroformate gave carbo-*p*-bromobenzyloxyglycine ethyl ester (m.p. 74°), which yielded a hydrazide (m.p. 148°) (found: C, 39.9; H, 3.5. $C_{10}H_{12}O_3N_3Br$ requires: C, 39.7; H, 3.85 per cent). Hydrogenation removed the protecting group from carbo-*p*-bromobenzyloxyglycine in the normal manner. All these materials have melting points higher than the corresponding carbo-benzyloxy compounds, and in general they crystallize with greater ease. Derivatives of other amino-acids are being prepared.

Other modifications of the carbobenzyloxy group are being examined, but meanwhile the use of *p*-bromobenzyl chloroformate may be found advantageous in peptide synthesis.

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¹ Bergmann, M., and Zervas, L., *Ber.*, **65**, 1192 (1932).

Epidermal Mitotic Activity and Oxygen Tension

THE effects of anaerobic conditions on mammalian epidermis were first studied by Medawar¹, who developed an elegant technique for showing that this tissue can survive by anaerobic glycolysis alone, but that cell movement and division depend on "respiratory activity in the narrow sense". These conclusions, which apply to the rabbit, have now been confirmed and elaborated in the case of the mouse. A technique has been devised, using standard Warburg equipment, for the maintenance of ear epidermis from adult male mice in a phosphate-buffered saline with added glucose.

Five fragments of ear epidermis (c. 2.5 mm. × 5.0 mm.) were immersed in 4 ml. saline medium in each flask, the side arm containing 0.016 mgm. colchicine in 0.04 ml. solution. The manometers were gassed with oxygen, nitrogen, or oxygen/nitrogen mixtures as required, and the flasks were maintained at 38° C. After one hour, when all the mitoses originally present were judged to have passed the meta-

phase, the colchicine was tipped into the main compartment. The incubation was then continued for a further four hours, when the tissue was fixed for sectioning. The results are shown in Table 1.

These figures indicate a direct linear relationship between epidermal mitotic activity and oxygen tension, and coincident observations on oxygen uptake (without colchicine) have confirmed that with increased oxygen tensions increased quantities of oxygen are absorbed (Table 2).

Table 2. $Q(O_2)$ of mouse-ear epidermis determined during 4 hr. incubation at 38° C. in phosphate-buffered saline with added glucose ($Q(O_2)$ = μ l. oxygen consumed/mgm. dry wt./hr.). Each figure is the average of 8 observations

Gas phase:		
80% N ₂ /20% O ₂	40% N ₂ /60% O ₂	100% O ₂
1.18 ± 0.05	1.52 ± 0.06	1.83 ± 0.07

Evidently a direct linear relationship also exists between oxygen consumption and oxygen tension.

Confirmation is thus provided for the theory already advanced that epidermal mitotic activity depends on the energy derived from the aerobic metabolism of glucose^{2,3}. If this is so, then by adding to the saline medium known catalysts of the tricarboxylic acid cycle, it should be possible to increase the oxygen consumption and hence the mitosis rate. The results of an experiment in which 0.02 M sodium glutamate was present in the saline medium are shown in Table 3.

Table 3. The influence of 0.02 M sodium glutamate on the numbers of mitoses arrested by colchicine in 4 hr. and on the $Q(O_2)$ of mouse-ear epidermis incubated at 38° C. in phosphate-buffered saline with added glucose and a gas phase of oxygen

Numbers of mitoses (5 observations)		$Q(O_2)$ (8 observations)	
without glutamate	with glutamate	without glutamate	with glutamate
6.7 ± 0.60	10.0 ± 0.75	1.83 ± 0.07	2.40 ± 0.13

It has already been established that glucose and oxygen exert their action on mitosis at a time immediately prior to the prophase, and that they are without effect once the prophase has begun⁴. This pre-prophase is evidently a critical time in the physiology of mitosis, and the name of 'antephase' has been suggested for it. The evidence so far available suggests that the energy required for mitosis must be built up in the antephase, and that once a division begins it can continue to completion in almost any circumstances short of the death of the cell. On one hand, the numbers of mitoses developing can be increased by stimulating the rate of energy production from glucose oxidation, while, on the other, they can be depressed by anaerobic conditions and by such known inhibitors of cellular metabolism as cyanide, fluoride, and dinitrophenol⁵. However, none of those substances which stimulates or depresses mitotic activity in this way appears to have any substantial effect on the course of a cell division once it has begun.

A full account of the techniques devised for this work and of experiments with catalysts and inhibitors will be published in due course.

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Table 1. The average numbers of mitoses arrested by colchicine in unit lengths (1 cm.) of sections (7 μ thick) of mouse ear epidermis after 4 hr. incubation at 38° C. in phosphate-buffered saline with added glucose. Each figure is the average of 10 observations

Gas phase:					
100% N ₂	80% N ₂ / 20% O ₂	60% N ₂ / 40% O ₂	40% N ₂ / 60% O ₂	20% N ₂ / 80% O ₂	100% O ₂
0.4 ± 0.16	2.2 ± 0.26	3.9 ± 0.27	5.5 ± 0.33	6.8 ± 0.34	8.3 ± 0.49

¹ Medawar, P. B., *Quart. J. Micr. Sci.*, **88**, 27 (1947).

² Bullough, W. S., *J. Exp. Biol.*, **26**, 83 (1949).

³ Bullough, W. S., *J. Endocrinol.*, **6**, 350 (1950).

⁴ Bullough, W. S., *Exp. Cell Res.*, **1**, 497 (1950).

⁵ Needham, J., "Biochemistry and Morphogenesis" (Cambridge, 1942).