

les 24 h après la mue aux 2ème et 3ème âges, les 48 h après la mue aux 4ème et 5ème âges, 1 à 4 jours après la mue chez l'imago, la cuticule non encore durcie (température des élevages: 35–38°C le jour, 20°C la nuit). Il n'a plus lieu lorsque des imagos sont maintenus à 100% d'humidité à partir du 5ème jour qui suit la mue imaginale. L'âge du parent maternel n'intervient pas dans le déterminisme de ce virage chromatique, qui est directement conditionné par le degré hygrométrique.

La livrée verte peut s'intensifier au cours de l'intermue; elle ne revire jamais au brun, à moins de chute importante de l'humidité ambiante. Ce retour au brun, continu et progressif, peut survenir à un moment quelconque de l'intermue; il est d'autant plus lent que le développement postembryonnaire est plus avancé. Il exige deux intermues lors du retour au climat sec de larves vertes du 2ème âge, est très partiel et tardif lors de ce retour chez les larves du 5ème âge, ne se fait plus jamais complètement chez l'imago.

Chez les imagos bruns, il existe une adaptation chromatique morphologique, se faisant progressivement, après le durcissement de la cuticule; lorsque les animaux sont placés sur fond noir, la teinte définitive est réalisée en une dizaine de jours. Les imagos bruns maintenus sur fond vert ne changent pas de couleur. Chez les animaux dont le verdissement a été induit par une humidité relative élevée, le maintien sur fond noir aboutit à l'assombrissement des ailes, des régions suboculaires et de deux taches sur le pronotum, la teinte générale restant verte. Il n'existe aucune adaptation chromatique chez les imagos grégaires.

Ces changements de couleur sont à rapprocher de la teinte de fond du biotope habituel; à l'humidité élevée correspond la teinte verte de la végétation (solitaires verts), à l'aridité la gamme de teintes de la végétation desséchée (solitaires jaunes, gris, noirs).

A la différence de coloration des imagos bruns et verts correspond une différence importante dans la résistance au jeûne suivant le degré hygrométrique. Les solitaires verts survivent en moyenne 140 h à 30% et 200 h à 95% d'humidité relative (température 30°C). Les solitaires bruns survivent respectivement 185 et 150 h, les grégaires 210 et 88 h; c'est chez ces derniers que le retentissement du degré hygrométrique sur le délai de survie est le plus fort. Des différences de résistance au jeûne allant dans le même sens existent entre les majoritaires verts et les minoritaires bruns élevés à l'humidité, ainsi qu'entre les majoritaires bruns et les minoritaires verts élevés au sec.

Dans le cas des animaux grégaires, des différences de résistance au jeûne par rapport aux solitaires n'existent que pour les souches maintenues dans une ambiance sèche; il y a lieu d'en conclure que le groupement seul ne suffit pas à déterminer une résistance plus élevée dans des conditions moins favorables.

Il résulte des faits exposés que l'état hygrométrique au cours du développement postembryonnaire prépare les individus majoritaires à la meilleure survie dans ces mêmes conditions; les minoritaires paraissent, au contraire, préparés à assurer la persistance de la population en cas de changement des conditions hygrométriques.

*Summary.* This investigation centres on the direct relationship between atmospheric humidity and the proportion of green and brown forms in *Locusta ph. solitaria*, on the ability of the brown forms to adjust their colours to the tints of their surroundings, and on the survival capacity of unfed *solitaria* and *gregaria* adults.

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## The Rate of Incorporation of Gaseous $^{14}\text{CO}_2$ into Brain Tissue Constituents

*Introduction.* Earlier, and more qualitative, indications for a significant incorporation of  $^{14}\text{CO}_2$  into organic tissue constituents<sup>1,2</sup> was recently confirmed and extended when BERL et al.<sup>3</sup> showed that the dicarboxylic acids glutamic and aspartic acid are rapidly labelled when  $\text{NaH}^{14}\text{CO}_3$  is infused into the carotid system in cats. However, neither the time course of the incorporation, nor the incorporation into tissue fractions other than part of the acid-soluble fraction was studied in the latter paper. There was thus no way to relate the findings to the rate of incorporation of  $^{14}\text{CO}_2$  into the volatile  $\text{CO}_2$  compounds ( $\text{HCO}_3^-$ ,  $\text{CO}_2$ ) of brain tissue, which has recently been studied in rats<sup>4</sup>.

The present work aimed at a description of the incorporation of  $^{14}\text{CO}_2$  into the main tissue fractions of rat brain tissue after exposure of the animals to a constant concentration of gaseous  $^{14}\text{CO}_2$  for time periods up to 120 min. The tissue fractions studied included the acid-labile, acid-soluble, protein, lipid and the nucleic acid fractions.

*Methods.* The experiments were performed on rats, weighing between 150 and 160 g. The rats were anaesthetized with Nembutal (4 mg/100 g i.p.) and allowed to breathe spontaneously. Each animal was exposed to a flowing gas mixture containing 17  $\mu\text{C } ^{14}\text{CO}_2/\text{l}$  but with a negligible carbon dioxide tension ( $\text{P}_{\text{CO}_2} < 0.5 \text{ mm Hg}$ ).

After exposure periods varying from 15 to 120 min, the brains were frozen *in situ* with liquid nitrogen and stored at liquid nitrogen temperature until analysis<sup>4</sup>.

In each experiment the acid-labile, the acid-soluble, the protein, the lipid and the nucleic acid tissue fractions were analysed with respect to their  $^{14}\text{C}$  content. The acid-labile compounds ( $\text{HCO}_3^-$  and  $\text{CO}_2$ ) were determined as described elsewhere<sup>4</sup>. The acid-soluble fraction was extracted in the cold with 5% trichloroacetic acid after homogenization of the tissue. The lipids were extracted from the washed precipitate, using a 2:1 mixture of chloroform-methanol. The nucleic acids were extracted at 90° in 5% trichloroacetic acid, and the protein residue was washed with acetone and ether and air-dried. The acid-soluble, the lipid and the nucleic acid tissue fractions were taken down to a small volume by evaporation. The evaporated samples, together with the protein and one untreated tissue sample were then digested in 1M Hyamine Hydroxide (Hyamine 10-X, Rohm and Haas) for 6–12 h at

<sup>1</sup> W. D. ARMSTRONG, J. SCHUBERT, and A. LINDENBAUM, Proc. Soc. exp. Biol. Med. 68, 233 (1948).

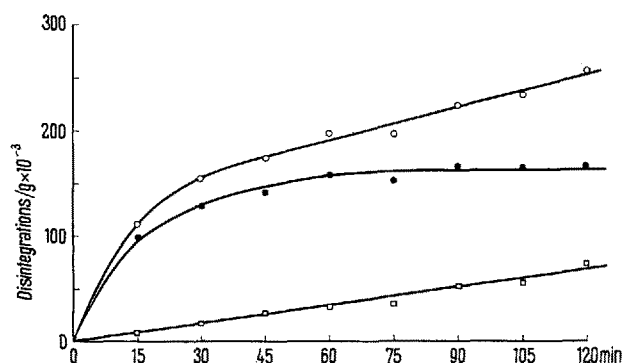
<sup>2</sup> H. L. KORNBERG, R. E. DAVIES, and D. R. WOOD, Biochem. J. 51, 351 (1952).

<sup>3</sup> S. BERL, G. TAKAGAKI, D. D. CLARKE, and H. WAELSCH, J. biol. Chem. 237, 2570 (1962).

<sup>4</sup> B. K. SIESJÖ, J. Physiol. 168, 59 P (1963).

65°C. The clear and only slightly coloured solutions were diluted with dioxane, and aliquots of the resulting solutions added to counting vials containing a scintillation mixture of PPO and POPOP in toluene<sup>6</sup>. The samples were then refrigerated before counting in a Panax liquid scintillation equipment.

**Results.** The results of the experiments can be summarized as follows (Figure). Upon the administration of a gas mixture with a constant concentration of  $^{14}\text{CO}_2$ , the total tissue concentration of radioactive  $\text{CO}_2$  increased roughly exponentially during the first 60 min, after that time the increase was roughly linear. The main part of the  $^{14}\text{CO}_2$  was recovered in the acid-labile  $\text{CO}_2$  fraction, and thus represented  $\text{HCO}_3^-$  and  $\text{CO}_2$ . The increase of radioactivity in this fraction was roughly exponential<sup>4</sup>, a steady state being reached after about 60 min. The organic fractions took up  $^{14}\text{CO}_2$  at a rate which was linear with time, the  $^{14}\text{CO}_2$  fixed in organic compounds after 15, 30, 60 and 120 min amounted to 9, 17, 21 and 35%, respectively, of the total radioactivity of the tissue. Of these organic fractions, the main part of the radioactivity was recovered in the acid-soluble fraction, while of the remainder only the proteins were labelled to a significant degree. Thus, the



The rate of incorporation of inspired  $^{14}\text{CO}_2$  into the brain tissue of rats (unfilled circles) as well as into the acid-labile (filled circles) and the acid-soluble (squares) fractions of the tissue. The difference between the curve depicting the 'total' incorporation (unfilled circles) and the curve representing incorporation into the acid-labile compounds ( $\text{HCO}_3^-$  and  $\text{CO}_2$ , filled circles) is the total incorporation into organic tissue constituents.

### Renal Excretion of Calcium-Disodium-Ethylenediaminetetraacetic Acid – A New Tubular Secretory Mechanism?

The rapid turnover of ethylenediaminetetraacetic acid (EDTA) in the organism, which, together with its metabolic inertia, is the cause of its very low toxicity, can be attributed chiefly to very rapid renal excretion. The first evidence that this substance is excreted by active secretion of the renal tubules, as well as by glomerular filtration, was the finding that its plasmatic clearance was the same as the clearance of diodrast<sup>1</sup>. We therefore undertook a detailed analysis of the mechanisms which participate in its excretion from the organism. Rats with a chronic urinary bladder fistula were anaesthetized and hydrated with 12% ethanol solution, the calcium-disodium salt of EDTA (50 mg/kg B.W. and inulin 25

labelling of the protein fraction at all times amounted to about 10% of the radioactivity which was fixed in organic compounds. During the time periods studied, labelling of the lipids and the nucleic acids was hardly significant.

**Discussion.** The results show that there is a rapid exchange between 'inorganic' C ( $\text{CO}_2$  and  $\text{HCO}_3^-$ ) and organic C in the acid-soluble compounds of the tissue. It is striking that within 15 min of exposure, about 10% of the total  $^{14}\text{CO}_2$  content in the tissue is organically fixed. The findings confirm and extend the recent observations of a rapid labelling of dicarboxylic acids from infused  $\text{NaH}^{14}\text{CO}_3$ <sup>3</sup>. It is tempting to assume that the major part of the  $\text{CO}_2$  fixed in our experiments has been incorporated into the dicarboxylic acids, and that the incorporation of these acids into proteins is responsible for the labelling of the protein fraction. It is proposed that the observations should be extended to a study of the incorporation of  $^{14}\text{CO}_2$  into different acid-soluble tissue compounds, since the present technique of administering the  $^{14}\text{CO}_2$  is more physiological and permits better quantitation than previous methods of administering radioactive bicarbonate by intraperitoneal, intravenous or intraarterial injections.

**Zusammenfassung.** Die  $^{14}\text{CO}_2$ -Fixation verschiedener Fraktionen des Rattengehirnes wurde untersucht. Die Ratten wurden 15–120 min in gasförmigem  $^{14}\text{CO}_2$  exponiert und in flüssigem  $\text{N}_2$  gefroren. Nach 15, 30, 60 und 120 min waren 9, 17, 21 und 35% vom Gesamt- $^{14}\text{CO}_2$  in der Gehirnschubstanz organisch gebunden. Der grösste Teil des organischen  $^{14}\text{CO}_2$  fand sich in säurelöslicher Fraktion, während ein kleinerer Teil (etwa 10%) aus der Proteinfraction isoliert werden konnte.

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*Institute of Animal Physiology, Babraham, Cambridge (England), September 30, 1963.*

<sup>5</sup> J. DULCINO, R. BOSCO, W. G. VERLY, and J. R. MAISIN, *Clin. chim. Acta* 8, 58 (1963).

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mg/kg) was injected and the cumulative excretion of both substances in the urine was studied for 4½ h and compared with the rate of excretion of sodium *p*-aminohippurate (PAH – a substance known to be excreted mainly by tubular secretion), administered in the same manner. Inulin was determined by means of  $\beta$ -indolacetic acid in a strongly acid medium<sup>2</sup>, EDTA by titration with  $\text{Bi}^{+++}$  in 0.01 *N* HCl using xylene orange as indicator<sup>3</sup>, PAH by diazotizing and coupling with *N*-1-naphthylethylenediamine<sup>4</sup>.

<sup>1</sup> H. FOREMAN, H. VIER, and M. MAGEE, *J. biol. Chem.* 203, 1045 (1953).

<sup>2</sup> A. HEYROVSKÝ, *Clin. chim. Acta* 1, 470 (1956).

<sup>3</sup> J. KÖRBL, R. PŘIBIL, and A. EMR, *Chem. listy* 50, 1440 (1956).

<sup>4</sup> H. W. SMITH, N. FINKELSTEIN, L. ALIMINOSA, B. CRAWFORD, and M. GRABAR, *J. clin. Invest.* 24, 388 (1945).