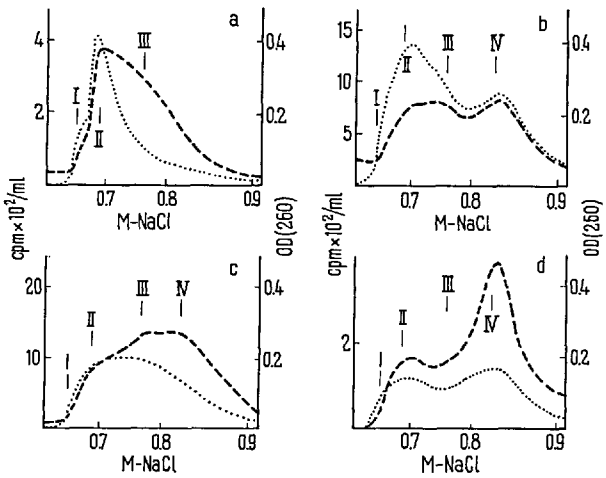
The RNA elution diagrams are presented in Figure a–d. The previously characterized RNA fractions are labelled as I (16s), II (23s)[5] and III (unstable, presumably messenger RNA[4]). An additional highly ^{32}P labelled RNA fraction IV, which elutes later than all other RNAs, is clearly recognizable in Figure b ('concentrated' cell culture) and d ('late' tumor cells), present in small amounts in c ('early' tumor cells) and absent in a ('diluted' cell culture). Since the applied chromatographic method separates the nucleic acids according to their chain length, the RNA fraction IV is considered to be of higher molecular weight. The region of its appearance in the elution diagram is comparable with the one of polio virus RNA[6].



Elution diagrams of RNA obtained from: a, 'diluted' cell suspension; b, 'concentrated' cell suspension; c, Ehrlich ascites tumor cells 'early'; d, Ehrlich ascites tumor cells 'late'. 0.8, 2.7, 2.7 and 2 mg of RNA were loaded onto the column in the experiments 'a', 'b', 'c' and 'd' respectively. For further details see text. ···· ultraviolet absorbancy at 260 mμ. ---- radioactivity, cpm-counts per min (please note that the radioactivity scale differs with every picture). The Roman numbers correspond to the following fractions on the diagram: I: 16 s, ribosomal RNA; II: 23 s, ribosomal RNA; III: unstable, presumably messenger RNA; IV: highly polymerized RNA described in the text (for additional information on fractions I–III see[3–5]).

The RNA from fraction IV is also more stable than the one from fraction III. The latter is completely broken whereas the former remains intact to 70% when incubated at 23°C for 60 min[4].

The synthesis of this RNA eluting last was observed also in other animal cells under unfavourable growth conditions, e.g. in human amnion cells during incubation in buffered saline, incomplete medium and in highly concentrated cell suspension. In tumor-bearing mice, this highly polymerized RNA was not only recognizable in neoplastic cells but also increased steadily in the liver until death.

On the other hand, no synthesis of this RNA was detectable by our methods in cells under optimal growth conditions. Investigations are under way to obtain information on the biological role of this RNA[7].

Zusammenfassung. Es wird der Einfluss von Stoffwechselbedingungen auf die RNS-Synthese in intakten Zellen *in vivo* und *in vitro* mittels ^{32}P-Markierung und einer säulenchromatographischen Fraktionierung der isolierten RNS untersucht.

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Laboratorium der Stiftung zur Erforschung der spinalen Kinderlähmung und der Multiplen Sklerose, Hamburg-Eppendorf (Germany), January 10, 1963.

[6] H. KUBINSKI and G. KOCH, Virology *17*, 219 (1962).

[7] This work was supported in part by the Deutsche Forschungsgemeinschaft and the Verein zur Erforschung und Bekämpfung der spinalen Kinderlähmung e.V., Bielefeld.

Accurate Determination of Total CO_2 in the Brain

All previous measurements of tissue carbon dioxide contents have been performed on excised tissues, in which *post mortem* changes might seriously have influenced the true *in vivo* levels of the labile carbon dioxide compounds. In order to avoid such changes, a method was developed which allows the determination of the total carbon dioxide content of tissues, frozen *in situ*. The results with this method indicate that the majority of the values reported earlier are too high and, further, that part of the difference is due to *post mortem* changes.

Methods. The experiments were performed on rats of the Sprague–Dawley strain which were anaesthetized with Nembutal (40–50 mg/kg body weight), tracheotomized and allowed to breathe spontaneously. The carbon dioxide tension of the blood was measured on samples drawn from a cannula in the femoral artery using the micromethod of SIGGAARD ANDERSEN et al.[1]. Immediately after the analysis, the brain was frozen *in situ* by plunging the whole animal into liquid nitrogen. When *post mortem* changes were to be investigated, the blood sampling preceded decapitation of the animal and rapid excision of the tissue, which was then placed in the liquid nitrogen. In some experiments, the period between the decapitation and the freezing of the tissue was deliberately prolonged so as to give delays of up to 10 min. In the group of animals analysed after quick-freezing, the brains were separated from the carcasses in the frozen state, using chisel and hammer. In all cases the supratentorial parts of the brain were used for the analyses.

The subsequent handling of the brain samples occurred in a Perspex box, manipulations of the samples being carried out with neoprene gloves. The box was continuously perfused with nitrogen gas which had previously been passed through devices for absorbing carbon dioxide and water vapour. The brain samples were transferred to a stainless steel mortar and, while still in liquid nitrogen, ground to a fine powder. Aliquots of the powder were added to special diffusion chambers mounted on Pyrex lids which had been blown and ground to fit commercial Conway 2-A units (Gallenkamp, London)[2]. The chambers contained a 5% trichloracetic acid solution, which could be stirred continuously. The amount of tissue added to the chambers was weighed by difference to the nearest tenth of a mg. 2 h were allowed for diffusion, the evolved carbon dioxide being captured in $0.2N$ $Ba(OH)_2$, which

[1] O. SIGGAARD ANDERSEN, K. ENGEL, K. JÖRGENSEN, and P. ASTRUP, Scand. J. clin. lab. Invest. *12*, 172 (1960).

[2] U. PONTÉN and B. K. SIESJÖ, to be published (1963).

was then titrated with 0.02*N* HCl in a stream of carbon dioxide-free nitrogen gas. Every third unit was run as a blank.

Results. It was found that all frozen tissue material required manipulation in the Perspex box. Thus, if brain tissue was fragmented in liquid nitrogen and transferred to the diffusion chambers while outside the box, irregular and high values were obtained. Manipulation in the box with absorption of only carbon dioxide did not prove sufficient since condensation of water vapour onto the frozen tissue samples also caused irregular values.

Two to five determinations of the total carbon dioxide content were made on each brain. Statistical calculation on 571 determinations in 161 groups, assuming that all measurements belonged to the same population, gave a coefficient of variation of 0.6%.

In order to compare values obtained after freezing the brain *in situ* with those obtained after decapitation and excision of the tissue, only those experiments were chosen in which the arterial carbon dioxide tension was between 35 and 45 mm Hg. Twenty-four such experiments were performed with decapitation of the animals and freezing of the tissue *after* excision (Figure 1).

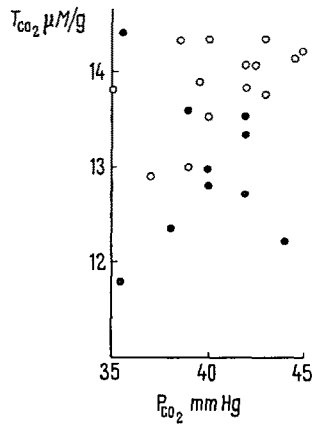

Fig. 1. Relation between arterial carbon dioxide tension and total carbon dioxide content in rat brain tissue. Nembutal anaesthesia and spontaneous respiration .The animals were decapitated and the tissue excised and placed in liquid nitrogen. Time between decapitation and immersion of tissue in liquid nitrogen either less (unfilled circles) or more than (filled circles) 2.5 min.

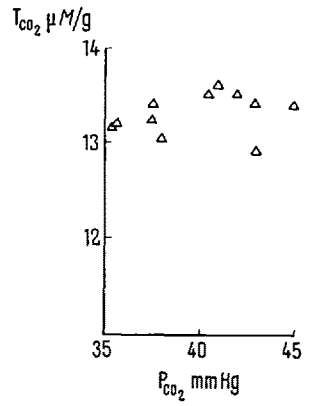

Fig. 2. Relation between arterial carbon dioxide tension and total carbon dioxide content in rat brain tissue after freezing the tissue *in situ* with liquid nitrogen. Nembutal anaesthesia and spontaneous respiration. Note the large difference in scatter of the values obtained after decapitation (see Figure 1) and freezing *in situ* respectively.

In Figure 2 are plotted the corresponding values obtained for the carbon dioxide content of the tissue after freezing the brain *in situ* by immersion of the whole animal in liquid nitrogen. It can be seen that the values spread much less than in the group of brains frozen *post mortem*. After quick-freezing there is also a much better correlation between the arterial carbon dioxide tension and the tissue carbon dioxide content[2]. The mean value for the tissue carbon dioxide content in the group with arterial carbon dioxide tensions between 35 and 45 mm Hg was 13.31 µMoles/g of wet weight. In no case did any value measured in the present series after *in situ* freezing exceed 14.0 µMoles/g.

Discussion. The present experiments show that reproducible and consistent values for the total carbon dioxide content of brain tissue can only be obtained if the tissue is frozen *in situ* and, further, that the frozen tissue samples must be treated with special precautions so as to exclude the disturbing influence of the carbon dioxide and the water vapour of ambient air.

The values reported previously for the total carbon dioxide content of rat brain tissues are 12.7[3], 14.1 and 15.1[4], 16.3 and 16.9[5] and 15.1[6] µMoles/g of tissue. All values except one are higher than those reported here. It is obvious that the difference is partly due to *post mortem* changes (see Results).

If it can be assumed that the true extracellular space of the brain is around 4%[7], the blood content after freezing *in situ* about 3%[8] and the amount of cerebro-spinal fluid about 9%[9], the total carbon dioxide content of the cell phase reduces to approximately 11.1 µMoles/g. Then, at a tissue carbon dioxide tension of 45 mm Hg, the amount of carbon dioxide dissolved is 1.3 µMoles/g[10] and the average bicarbonate concentration thus around 9.8 µMoles/g[11–13].

Zusammenfassung. Methode zur Bestimmung des Gesamtkohlensäuregehalts im Gehirngewebe mit einem Variationskoeffizienten von 0.6%. Versuche mit dieser Methode zeigen, dass die Analysenwerte ohne Einfrierung des Gehirns *in situ* zu hoch liegen und schlecht reproduzierbar sind.

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[13] *Acknowledgments*: This investigation was supported by grants from the Medical Faculty of Lund and by the Swedish Medical Research Council.