3 proteolytic enzymes, chymotrypsin, trypsin or papain led to significant lowering of the HA-I titer (Table II). Human placental alkaline phosphatase preparations were found to inhibit agglutination by PR8-influenza A virus of human erythrocytes as effectively as agglutination of chicken red blood cells. The antigen-antibody precipitate formed by reacting purified placental alkaline phosphatase with specific antiserum exhibited alkaline phosphatase activity as well as HA-I titer of 184 units per unit of enzyme activity.

Certain malignant tumors and HeLa cells in tissue culture contain an alkaline phosphatase with physical, chemical and immunologic properties that closely resemble the placental forms of the enzymes[12,13]. $HeLa_{65}$ cell alkaline phosphatase (purified 220-fold) exhibited viral hemagglutination inhibitory potency. The present investigation[14] indicates that the high molecular weight variants of placental alkaline phosphatase are potent inhibitors of influenza virus hemagglutination. The inhibitory capacity of the enzyme seems to be dependent both upon sialic acid residues and molecular size.

Résumé. La phosphatase alkaline du placenta humain est un sialyglycoenzyme qui inhibe l'hémagglutination (HA) produite par le *Virus influenzae* A (PR-8). L'enzyme de poids moléculaire élevé donne un titre HA-I beaucoup plus grand que les enzymes de poids moléculaire inférieur L'élimination de l'acide sialique de l'enzyme par traitement à la neuraminidase ou par oxydation des carbohydrates avec du périodate ou par protéolyse amènent une réduction prononcée du titre de HA-I.

N. K. Ghosh, A. Rukenstein, C. Labowsky and R. P. Cox

Departments of Medicine, Pharmacology and the Stella and Charles Guttman Laboratory for Human Pharmacology and Pharmacogenetics, New York University Medical Center, 550 First Avenue, New York (N.Y. 10016, USA), 5 October 1973.

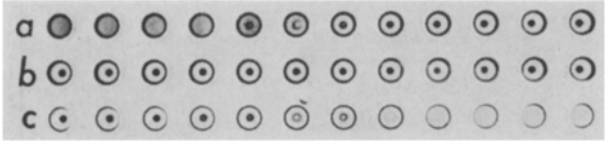

Fig. 2. Effect of placental alkaline phosphatase on hemmagglutination caused by PR8-influenza A virus. a) Serial dilutions of 32 units of virus plus red cells (hemagglutination is observed up to the 4th dilution or 4 units of virus) b) Red cells alone in buffered saline (control) c) 8 units of virus previously incubated with serial dilutions of placental alkaline phosphatase and red cells (hemagglutination-inhibition is observed through the 7th well or a dilution of 1:128 of the enzyme).

[12] N. A. Elson and R. P. Cox, Biochem. Genet. *3*, 549 (1969).
[13] W. H. Fishman, N. R. Inglis, S. Green, C. L. Anstiss, N. K Ghosh, A. E. Reif, R. Rustigian, M. J. Krant and L. L. Stolback, Nature, Lond. *219*, 697 (1968).
[14] Supported by NIH Research Grant No. GM11508 and Career Scientist Award to R.P.C. by the Health Research Council of the City of New York. N.K.G. is grateful to Dr. P. Calabresi for his help and support.

The Kinetics of Cerebral RNA Synthesis in Relation to the Route of Injection

Many kinetic studies exist on the metabolism of cerebral RNA in the living animal. These have generally utilized radioactive RNA precursors such as inorganic phosphate or nucleotides which have been injected subcutaneously[1-3] intracranially[4,5] or into the circulatory system[6-8]. After various time intervals animals are killed and the extent of isotopic incorporation into RNA is related to the amount of radioactivity remaining within the acid soluble pool. In the search for a relation of cerebral macromolecule synthesis to brain function, relatively minor differences in the rate of RNA synthesis in animals under differing experimental conditions have been reported[2,3,6].

The kinetics of the synthesis of labelled cerebral RNA from ^{3}H-uridine administered by various routes in the young chick are reported. The data suggests that rates of cerebral RNA synthesis are not readily determinable, and that a variety of parameters must be taken into account.

Methods and materials. 1–4-day-old chicks were injected with 5-^{3}H-uridine (27.1 Ci/mmol) in 3 different ways: 1. by s.c. injection into the scruff of the neck of 50 μCi ^{3}H-uridine. 2. by intracardiac injection of 50 μCi ^{3}H-uridine. 3. by intracranial injection of 5 μCi ^{3}H-uridine into the right cerebral hemisphere. After various time intervals, chicks were decapitated and the right cerebral hemisphere rapidly dissected out. Hemispheres were then homogenized in 5 ml 0.32 *M* sucrose at 0 °C, and this homogenate was centrifuged at 1000 *g* for 10 min. 0.5 ml 50% (w/v) TCA was then added to the resulting cytoplasmic supernatant and the precipitate was resuspended in cold 5% TCA. Both supernatant and precipitate were then centrifuged at 0 °C and 1000 *g* for 10 min. Unincorporated radioactivity was assayed in samples of the supernatant from the TCA-treated cytoplasmic fraction. The precipitates were washed twice more with cold 5% TCA, once with ethanol, and were then incubated for 2 h at 37 °C in 2 ml 0.1 *N* NaOH. This suspension was then brought to a pH below 3.0 with perchloric acid and the precipitate of protein and DNA was removed by centrifugation. Radioactivity within the supernatants containing hydrolysed RNA was determined.

The proportion of radioactivity remaining in nucleotides in the acid-soluble TCA supernatant was assayed by absorption on to charcoal. 1 ml aliquots of these supernatants were taken and made up to 10 ml in 0.1 *N* HCl. Around 5 mg of charcoal (acid washed) were stirred into each sample which was then centrifuged. Under these conditions, uridine and its phosphorylated nucleotides bind to the charcoal[9,10]. 1 ml samples of the supernatants were counted in order to determine non-nucleoside,

[1] D. H. Adams, J. Neurochem. *12*, 783 (1965).
[2] H. D. Cohen and S. H. Barondes, J. Neurochem. *13*, 207 (1966).
[3] J. W. Zemp, J. E. Wilson, K. Schlesinger, W. O. Boggan and E. Glassman, Proc. natn. Acad. Sci. USA *55*, 1423 (1966).
[4] S. C. Bondy, J. Neurochem. *13*, 955 (1966).
[5] K. von Hungen, H. R. Mahler and W. J. Moore, J. biol. Chem. *243*, 1415 (1968).
[6] R. E. Bowman and D. A. Strobel, J. comp. physiol. Psychol. *67*, 448 (1969).
[7] J. Engel and F. Morrell, Expl. Neurol. *26*, 221 (1970).
[8] H. Pakkenberg and R. Fog, Expl. Neurol. *36*, 405 (1972).
[9] C. H. Fiske, Proc. natn. Acad. Sci., USA *20*, 25 (1934).
[10] R. K. Crane and F. Lipmann, J. biol. Chem. *201*, 235 (1953).

non-nucleotide radioactivity. Nucleosides and nucleotides were then eluted from the charcoal precipitate with 10 ml of an aqueous solution containing 70% (v/v) ethanol and 5% (v/v) of aq. NH_3 (sp. gr. 0.88). 1 ml samples of the supernatant obtained after recentrifugation were neutralized with conc. HCl, and counts within nucleosides and nucleotides were determined.

Results and discussion. The persistence of radioactivity within uridine derivatives was examined after administration of ^{3}H-uridine by various routes (Figure 1). After intracranial injection, the TCA soluble pool retained a high proportion of total counts as uridine derivatives, for at least 6 h. Subcutaneous or intracardiac injection resulted in a very steep decline of counts retained as nucleosides or nucleotides. The major part of acid-soluble radioactivity reaching the cerebral hemisphere after s.c. injection of ^{3}H-uridine was non-nucleotide in nature at all time points examined. It may be that intracardiacally or s.c. injected isotope reaches the liver and is rapidly metabolized to other products. Intracerebrally administered uridine may be taken up and retained by cerebral cells which are unable to catabolize uridine beyond the initial nucleoside cleavage to uracil[11].

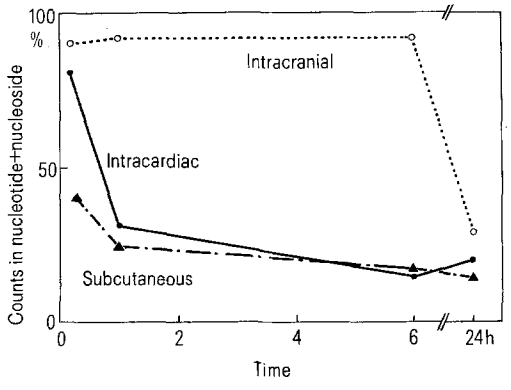

Fig. 1. Proportion of radioactivity that is absorable by charcoal, of the total acid soluble radioactivity found within the right cerebral hemisphere of the chick. Determinations were made at various times after administration of ^{3}H-uridine by several routes. ▲---▲, subcutaneously; ●—●, intracardiacally; ○---○, intracerebrally.

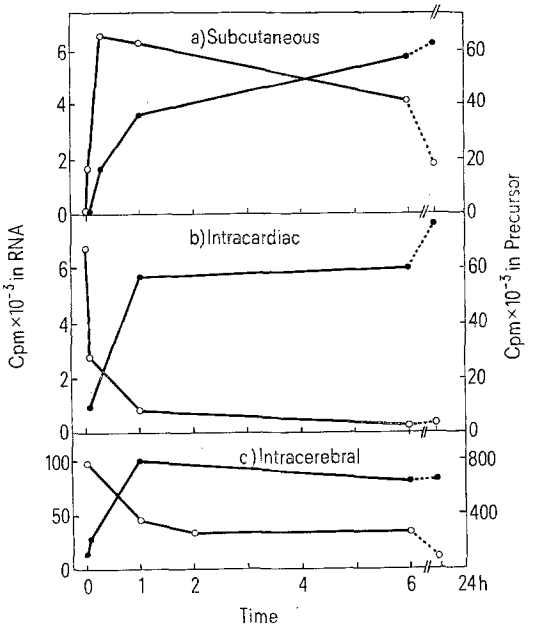

Fig. 2. RNA synthesis in chick right cerebral hemisphere following injection of ^{3}H-uridine: a) subcutaneously; b) intracardiacally; c) intracerebrally. ●—●, counts/min in RNA per cerebral hemisphere ○—○, counts/min in acid soluble nucleoside + nucleotide per cerebral hemisphere.

The proportion of non-charcoal-binding radioactivity that was not present as ^3H_2O was determined by lyophilization of samples and re-dissolving of residues in water, for assay of their remaining radioactivity. In samples where the amount of non-charcoal binding radioactivity was significant, 69–76% of this was volatile. Due to dilution with tissue water, such tritiated water has a very low specific activity and cannot contribute to the synthesis of new radioactive species to a major extent.

The kinetics of RNA synthesis varied considerably with the mode of precursor administration (Figure 2). Subcutaneous injection resulted in a rather slow rise of acid-soluble RNA precursors and a persisting nucleoside-nucleotide pool. Radioactive RNA synthesis took place at a decreasing rate for several hours. On the other hand, intracardiac or intracerebral injection of ^{3}H-uridine resulted in an initially high soluble nucleoside-nucleotide pool followed by a relatively rapid decline. In these cases the period of synthesis of labelled RNA appeared short and a maximum specific activity of RNA was reached around 1 h after precursor injection.

These data suggest that the expression of radioactivity within RNA in relation to simultaneously determined total label within the acid soluble pool is not very useful. The total extent of radioactive RNA synthesis may be more closely related to the integral of the levels of the nucleoside-nucleotide pool determined at a series of prior times.

The major purpose of this note is to point out that different means of isotope administration result in highly varied kinetics of radioactive RNA synthesis within the brain. The rate of conversion of the ^{3}H-uridine precursor to non-nucleoside, non-nucleotide substances is also determined by the site of isotope injection. For this reason, the radioactivity within the total acid-soluble fraction may bear little relation to the amount of label within chemicals directly associated with the path of RNA synthesis[12].

Zusammenfassung. Nachweis, dass die RNS-Synthese im Gehirn sich nach der Art der Injektion der radioaktiven Substanz richtet, während die Umsatzgeschwindigkeit von ^{3}H-Uridin in nicht-nukleotiden Substanzen ebenfalls von der Injektionsmethode abhängig ist.

P. C. MARCHISIO[13] and ST. C. BONDY

University of Colorado Medical Center, 4200 East 9th Avenue, Denver (Colorado 80220, USA), 25 September 1973.

[11] A. F. HOGANS, G. GUROFF and S. UDENFRIEND, J. Neurochem. *18*, 1699 (1971).

[12] Supported by National Institutes of Health Grant No. NS09603 and the Foundations' Fund for Research in Psychiatry, Grant No. 70-487. STEPHEN C. BONDY is recipient of National Institutes of Health Research Career Development Award No. NS49945.

[13] Present address: Department of Human Anatomy, University of Torino, Torino, Italy.