compounds, however, the value of certain modifications of structure in producing desired biological activity has been indicated. In view of the established marked antiviral activity of certain nucleosides it would appear that investigation of the potential antiviral activity of other nucleoside derivatives is warranted[6].

[6] This investigation was supported by grants from the National Cancer Institute of the U.S. Public Health Service (Nos. CA-05262 and CA-08109).

Zusammenfassung. Der virushemmende Effekt sowie die zytotoxische Aktivität gewisser Purine und Pyrimidin-Nukleoside gegen *Herpes-simplex*-Virus bzw. Kulturen von BSC-1-Zellen werden beschrieben.

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PRO EXPERIMENTIS

A Quantitative Evaluation of the Non-Enzymatic Conversion of Glutamine to Ammonia[1]

Ammonia is an end-product of both the enzymatic and non-enzymatic degradation of glutamine. In studying glutaminase activity it is not possible to distinguish between the ammonia formed by enzymatic and non-enzymatic reactions without performing substrate blanks. In previous studies[2,3] it became obvious that the optimum conditions required for rat renal glutaminase caused an appreciable non-enzymatic degradation of glutamine. Therefore a study was made of the rate of non-enzymatic ammonia production from glutamine with particular reference to the conditions which prevail when glutaminase activity is being assayed.

Ammonia was measured by the CONWAY microdiffusion technique[4]. The sample was alkalinized in the outer well of the unit by 1 ml of saturated potassium carbonate and the ammonia was trapped in the centre well by 1 ml of borate buffer. Except where otherwise stated, ammonia diffusion was allowed to take place at room temperature for 2 h.

The solid line in Figure 1 shows a typical recovery curve obtained with preformed ammonia. In this case the 10 μ equivalents of ammonia were recovered after 60 min. The broken line shows the rate of ammonia recovery when 120 μmoles of glutamine are added to the outer well in addition to the 10 μ equivalents of ammonium chloride. It will be noted that the curves separate after 5 min and that ammonia is being recovered from glutamine even after 3 h. These results show that when preformed ammonia is being analysed titration time is not critical after complete recovery but if glutamine is present in the sample titration time must be constant if the rate of degradation of glutamine is not known.

In glutaminase studies glutamine will usually be at 3 different temperatures for a significant period of time. The rate of glutamine degradation at these temperatures was then determined. For this type of experiment 120 μmoles of glutamine in a volume of 0.5 ml were put in sealed test-tubes with 1 ml of saturated potassium carbonate at various temperatures. At different time intervals a sample was removed from the incubator, quickly brought to room temperature and then poured into the outer well of the CONWAY unit. Ammonia absorption was allowed to take place for 2 h, which was more than sufficient time for diffusion of the largest amount of ammonia which was produced in these experiments. By bringing all samples to room temperature means that further degradation should be similar irrespective of the previous temperatures and that the rate of ammonia diffusion, which is temperature dependent[4], should be approximately equalized in all samples. The amount of ammonia recovered when 120 μmoles of freshly prepared glutamine were allowed to remain in the outer well for 2 h was subtracted from the values obtained. The corrected values are reported in Figure 2. It is obvious that at this high pH there is a considerable degradation of glutamine even at 0 °C. As the temperature was elevated there was an increase in the rate of degradation.

The effect of phosphate at various pH values and at 2 different temperatures is shown in Figures 3 and 4. At 20 °C (Figure 3) raising the pH from 5–10, in the absence of phosphate, increased the ammonia production by 50%. In the presence of 100 μmoles of phosphate a similar increase in the pH caused a 3-fold rise in ammonia production. This means that the effect of phosphate on the rate of glutamine degradation was greater at higher than at lower pH values. On the other hand, at 37 °C (Figure 4) phosphate had a similar effect on the rate of glutamine degradation at all pH values studied. However, at this higher temperature a rise in pH increased ammonia production more than it did at 20 °C. In the absence of phosphate an increase in the pH value from 5–10 caused a 50% rise in ammonia production at 20 °C and a 3- to 4-fold rise at 37 °C. Intermediate temperatures resulted in ammonia production between those values. Phosphate is normally employed in the assay of glutaminase 1. Due to its accelerating effect on non-enzymatic degradation of glutamine, even at 20 °C, at high pH values it will result in significant degradation of glutamine in the presence of potassium carbonate in the CONWAY unit.

All of the results reported here were obtained with 120 μmoles of glutamine. However, linear results were

[1] This work was supported by a grant from the Medical Research Council of Ireland.

[2] D. J. O'DONOVAN and W. D. LOTSPEICH, Nature *212*, 930 (1966).

[3] D. J. O'DONOVAN and W. D. LOTSPEICH, Enzymologia *35*, 82 (1968).

[4] E. J. CONWAY, *Microdiffusion Analysis and Volumetric Error* (Crosby Lockwood, London 1958).

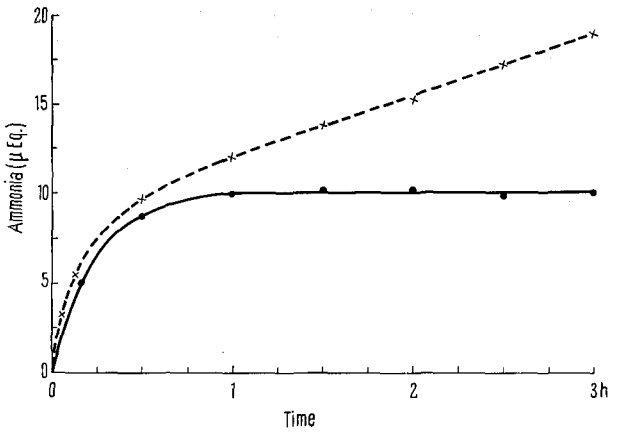

Fig. 1. 1 ml of saturated potassium carbonate and 0.5 ml of sample were placed in the outer well of the CONWAY unit. The sample consisted of 10 μ Eq. NH_4Cl (•–•–•) or 10 μ Eq. NH_4Cl + 120 μmoles glutamine (×–×–×). The rate of ammonia diffusion was detected by titrating the 1 ml of borate buffer in the centre well with 0.01 *N* HCl.

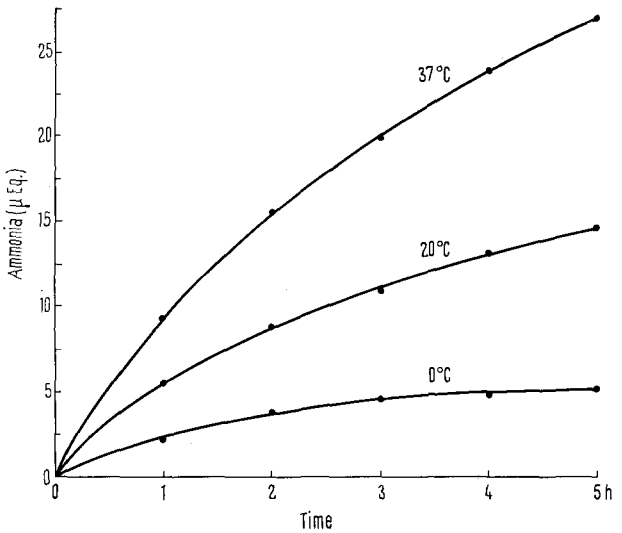

Fig. 2. 1 ml of saturated potassium carbonate and 0.5 ml of 0.24 *M* glutamine were put in sealed test-tubes for the times and temperatures indicated. They were then brought to 20 °C, transferred to the outer well of the unit and diffusion was allowed to take place for 2 h.

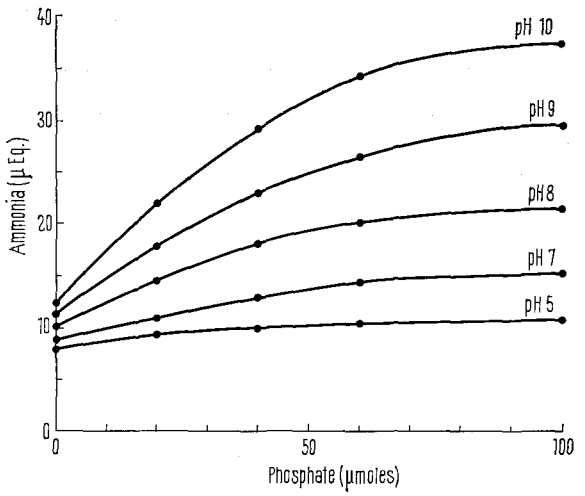

Fig. 3. 0.5 ml of 0.24 *M* glutamine and 0.5 ml of 0.2 *M* buffer were put in sealed test-tubes for 5 h at 20 °C. They were then transferred to the outer well of the unit and diffusion was allowed to take place for 2 h. The phosphate was varied by replacing equal volumes of the non-phosphate buffers by phosphate at a similar pH value.

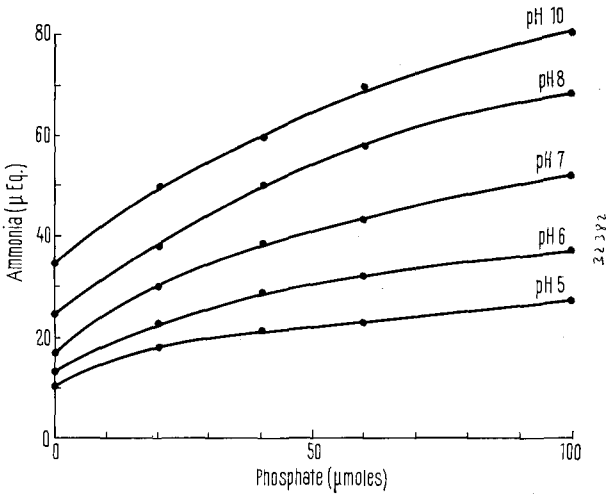

Fig. 4. Conditions were similar to Figure 3 except that incubation in the sealed test-tubes was at 37 °C. The temperature of the samples was quickly lowered to 20 °C before transference to the CONWAY unit.

found with a stepwise lowering of the glutamine to 12 μmoles and when the time, temperature, pH and phosphate concentrations were varied.

On account of the number of variables involved and because of the complexity of the interactions of some of these variables it is difficult to construct a nomogram. Indeed a nomogram would not be complete without including other variables such as arsenate, methyl arsenate, bicarbonate, and borate[5]. We only concerned ourselves with variables existing during glutaminase assay. The results here were obtained with freshly prepared glutamine and if a glutamine solution is stored and re-heated to get into solution again the values obtained will be increased. The data focus attention on the fact that when ammonia is assayed by alkalinization, to release it in the gaseous form for trapping in acid, glutamine present in the sample will be degraded to ammonia.

Zusammenfassung. Das Verhältnis nicht-enzymatischer Verwandlung von Glutamin zu Ammoniak wird gesteigert, wenn pH, Temperatur und Phosphatmenge vermehrt werden. Bei alkalischer Probe bemerkt man eine Verringerung von Glutamin.

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Department of Physiology, University College, Galway (Ireland), 10 July 1968.

[5] J. B. GILBERT, V. E. PRICE and J. P. GREENSTEIN, J. biol. Chem. *180*, 209 (1949).

[6] Miss G. W. WARNER was a recipient of a Medical Research Council of Ireland summer student fellowship during the performance of some of this work.