

IR-4B', we were able to show by paper chromatography the presence of small amounts of *d*(-)-glutamic acid which were not due to racemization.

In order to investigate whether this *d*(-)-glutamic acid arose from ordinary food proteins, the urine of starved rats was analysed. *d*(-)-Glutamic acid could readily be shown to be present, employing the procedures described above. The same result was obtained with human urine. We are now trying to determine differences in the *d*(-)-glutamic acid content of urine from patients with different diseases.

Since we have found *d*(-)-glutamic acid in the urine of starving humans and rats, it may well be that account must be taken of the presence of 'unphysiological' *d*-amino-acids in ordinary metabolism; this would seem to us to explain the so far ambiguous role of *d*-amino-acid oxidases.

Details of this work will be published in the *Zeitschrift für Naturforschung*.

GÜNTHER HILLMANN
ANNELIESE HILLMANN-ELIES
FRIEDRICH METHFESSEL

Chemisches Laboratorium,
Medizinische Universitätsklinik,
Tübingen.

¹ "Advances in Proteinchemistry", 4, 362. "Annual Review of Biochemistry", 15, 260.

² Kögl, F., *Experientia*, 5, 173 (1949).

³ Kögl, F., Barendregt, T. J., and Klein, A. J., *Nature*, 162, 732 (1948).

⁴ Ratner, S., *J. Biol. Chem.*, 152, 559 (1944).

⁵ Ayengar, P., and Roberts, E., *J. Biol. Chem.*, 197, 454 (1952).

Influence of Carbon Dioxide on the Toxicity of Ammonia

RIVERS polluted with sewage effluent may contain low concentrations of dissolved oxygen at places where the concentrations of ammonia and carbon dioxide are relatively high. It is known that both ammonia and carbon dioxide are toxic to fish in high enough concentration, and that they are rendered more toxic by lowering the concentration of dissolved oxygen^{1,2}. A study of the toxicity to fish of water containing both these substances when the concentration of dissolved oxygen was low, but not lethal, was thought desirable.

Sufficient ammonium chloride to give concentrations equivalent to 0, 15 and 30 parts of nitrogen per million was added to tap water at Garston, Watford. A mixture of oxygen, nitrogen and carbon dioxide was blown through the solutions to maintain the concentration of dissolved oxygen as close as possible to 4.41 p.p.m. and to give six concentrations of carbon dioxide, in the range 0-240 p.p.m. in each concentration of ammonium chloride; the temperature was maintained at 17.5° C. Ten yearling rainbow trout were placed in each of the eighteen solutions; and their periods of survival were observed. Each test was ended after 12 hr. even if some fish were still alive.

In the absence of ammonia, carbon dioxide was not toxic to the fish within 12 hr. at concentrations up to 30 p.p.m.; 60 p.p.m. carbon dioxide was toxic, however, and the periods of survival decreased as the concentration was increased above this level (Fig. 1). The solution containing ammonium chloride equivalent to 30 p.p.m. nitrogen was markedly toxic when no carbon dioxide was added; but as the concentration of carbon dioxide was increased to 30 p.p.m. the periods of survival lengthened. At concentrations

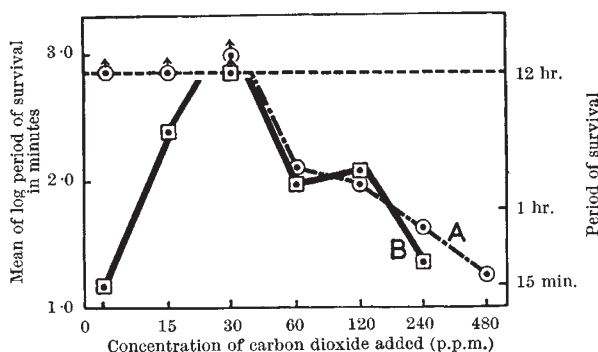


Fig. 1. Toxicity of carbon dioxide and ammonia to rainbow trout. A, solutions containing no ammonia. B, solutions containing ammonium chloride equivalent to 30 p.p.m. nitrogen

of carbon dioxide between 60 and 240 p.p.m. all solutions were toxic, and there was little difference between the concentration-survival-time curve for carbon dioxide with ammonia and the curve for carbon dioxide alone. Very similar results were obtained with ammonia solutions containing 15 p.p.m. nitrogen.

The results show that the presence of carbon dioxide up to a concentration within the range 15-60 p.p.m. reduces the toxicity of ammonia solutions; higher carbon dioxide concentrations are themselves toxic. It has been shown^{1,3} that the toxicity of ammonia solutions to fish is influenced by the pH value of the water, because less of the highly toxic un-ionized ammonia and more of the much less toxic ammonium ion is present at a low pH value. The pH values determined during the experiments with ammonia solutions fell with increasing concentrations of carbon dioxide from about 7.91 when none was added to 7.4 with 30 p.p.m. carbon dioxide and to about 6.9 with 120 p.p.m. carbon dioxide. The calculated amounts of un-ionized ammonia in these solutions were 1.08, 0.26 and 0.10 p.p.m. nitrogen respectively. It is virtually certain, therefore, that the lowering of the pH value is a principal, if not the only, cause of the reduction of the toxicity of the ammonia solutions by the addition of carbon dioxide.

This communication is published by permission of the Ministry of Agriculture and Fisheries and the Department of Scientific and Industrial Research.

J. S. ALABASTER

Ministry of Agriculture and Fisheries,
Whitehall, London.

D. W. M. HERBERT

Water Pollution Research Laboratory,
Watford, Herts.
May 10.

¹ Downing, K. M., and Merckens, J. C. (in preparation).

² Wells, M. M., *Biol. Bull., Woods Hole*, 25, 221 (1915).

³ Wührmann, K., and Woker, H., *Schweiz. Z. Hydrol.*, 11, 210 (1948).

Ergothioneine in the Equine Ampullar Secretion

A STRIKING feature of semen and of the reproductive organs in mammals is the diversity in chemical composition and secretory function encountered among different species¹. Recent studies on the occurrence of ergothioneine provide a good example of this behaviour. This sulphur-containing nitrogenous base, which has been isolated in a crystalline state from the boar vesicular secretion² and which has been