Australia Antigen: Detection and Transmission in Shellfish

Abstract. *Australia antigen was found in clams contaminated by drainage of untreated sewage from a coastal hospital. In closed-system aquariums, the antigen was ingested by clams and transmitted to previously uninfected clams. In open-system aquariums, the titer of Australia antigen decreased with time, suggesting viral concentration rather than replication.*

Epidemiological studies suggest a causal relationship between shellfish ingestion and viral hepatitis (*1*). Australia (Au) antigen is a marker of type B hepatitis virus. Using Au antigen, we studied the relation between clams and type B hepatitis virus. Partially purified Au antigen was prepared by sucrose gradient ultracentrifugation of Au-positive serum. The Au antigen was detected by immunodiffusion (*2*) and by liquid and solid phase radioimmunoassays (RIA) (*3*).

The antigen fraction was added to serial dilutions of homogenized pooled samples of normal clam tissues. From 68 to 110 percent of Au antigen added was recovered from clam tissue in vitro, an indication that Au antigen is not destroyed by clam tissue.

Approximately 500 clams were harvested from 21 areas along a part of the Atlantic Coast of Maine. These areas were closed to commercial clamming because of heavy bacterial pollution. Included were ten clam beds polluted by drainage of untreated sewage from coastal hospitals. Clams from each of the 21 areas were shelled, pooled, homogenized, and examined for Au antigen by RIA. From 20 to 24 pooled clams from one area were unequivocally positive for Au antigen on three separate occasions during a 2-month period, as determined by immunodiffusion as well as RIA. Unfortunately, subtyping of Au antigen was not performed. This particular clam bed has been closed to commercial clamming for more than 5 years and is polluted by untreated sewage directly draining from a small coastal community hospital. During the 3 months preceding this study, two individuals with Au-positive hepatitis were patients in this hospital. No clams from the 20 other areas revealed Au antigen when tested by RIA.

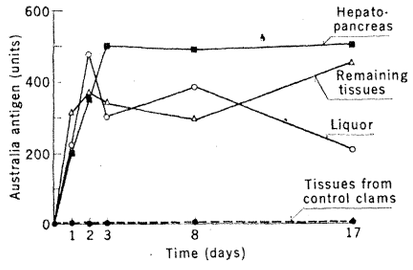

Fig. 1. Australia antigen concentration in clam tissues after addition of concentrated Au antigen (0.1 ml) to a closed-system aquarium on day zero. Normal serum was administered to control clams in a separate aquarium. The clams were removed from the tanks at 1 to 17 days. One unit equals the numbers of counts per minute per milliliter corrected for radioactivity of control tissue. Antigen or normal serum was added at time zero.

Laboratory studies were performed in closed-circuit, temperature-controlled aquariums (22°C). The Au antigen (subtype adw+, y−) was partially purified and concentrated by ultracentrifugation of 96 ml of Au-positive serum at 78,000*g* for 16 hours. The pellets were resuspended in one-tenth of their original volume in 0.1*M* NaCl, 0.01*M* tris-HCl, 0.001*M* ethylenediaminetetraacetic acid (sodium salt), *p*H 7.4 (TSE). The resuspended material was centrifuged over a cushion of 10 percent sucrose in TSE solution at 78,000*g* for 4 hours. The final pellet was suspended in TSE (one-tenth of original serum volume) checked for antigenicity, and stored at 4°C. Partially purified, concentrated Au antigen was placed in or near the afferent siphon of 15 to 25 cherrystone (*Mercenaria mercenaria*) clams that were obtained from a commercial supplier. The clams were kept in natural seawater and sampled from 1 to 17 days later; each sampled clam was separated anatomically into clam liquor, hepatopancreas, and remaining tissues. The clam liquor is the fluid collected when clams were opened before the tissue was dissected. Hepatopancreas included almost all of the digestive tract. Each fraction was homogenized. All specimens were coded and tested by RIA and immunodiffusion. Clams treated similarly with normal (Au-negative) serum served as controls. Progressive increase in titer of Au antigen occurred through the third day in all infected tissues (Fig. 1) with the greatest antigen concentration in the hepatopancreas.

After exposure to Au antigen for 4 days, clams were removed from the tanks and washed; then 2 to 4 clams were placed in closed-circuit aquariums with 10 to 20 previously uninfected clams. Tissues from previously uninfected clams were serially sampled at intervals from 1 to 20 days; they were separated into clam liquor, hepatopancreas, and remaining tissues; and the fractions were examined by RIA. Control clams were treated as before with normal serum. Australia antigen was detected in all previously uninfected clams throughout the experiment (Fig. 2). The rising titer suggests relative greater concentration in the hepatopancreas.

The question arises as to whether these data represent concentration of virus or replication. The following experiment suggests that concentration of virus may be the predominant mechanism. From 12 to 24 clams infected with Au antigen were placed in an open, circulating seawater system, and the effect of time on Au antigen concentration was determined. In all tissues, the titers of antigen progressively decreased, and antigen was no longer detectable in previously infected clams by day 12. Previous experiments concerning viruses and shellfish were performed with poliovirus as a model (*4*) and revealed concentration of the virus on the pseudomucus that lines the digestive tract, without evidence of viral multiplication.

Clams apparently filter and concentrate Au antigen in the water. The antigen may be transmitted from one clam to another. Our studies support epi-

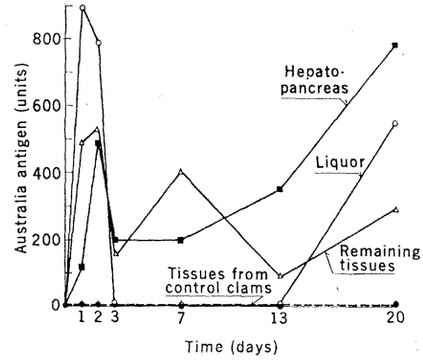

Fig. 2. Ten clams were exposed to concentrated Au antigen (0.1 ml) in a closed-system aquarium. Four days later, two "infected" clams were placed in a closed-system aquarium with 14 uninfected clams; these were sampled in pairs at 1 to 20 days. Normal serum was administered to control clams in a separate aquarium. Antigen or normal serum was added at time zero.

demiological observations linking clams as possible vectors in hepatitis and suggest that, under special circumstances, shellfish may serve as a reservoir for Au antigen and type B hepatitis virus.

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5. We thank the Department of Sea and Shore Fisheries of the State of Maine and the Mount Desert Island Biological Laboratories for cooperation. Supported by PHS grants AM 05384, AM 70228, CA 06551, CA 06927, and RR 05539 and NIH contract 70-2234 and an appropriation from the Commonwealth of Pennsylvania.

4 September 1973; revised 23 October 1973

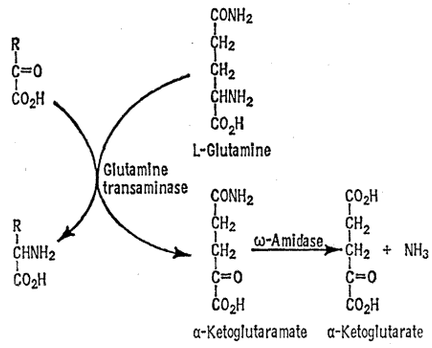
α-Ketoglutaramate: Increased Concentrations in the Cerebrospinal Fluid of Patients in Hepatic Coma

Abstract. *α-Ketoglutaramate, a deaminated metabolite of glutamine not previously identified in biological tissues, was measured in the cerebrospinal fluid of human subjects and found to be increased three- to tenfold in patients with hepatic coma. When perfused into the cerebral lateral ventricles of rats, α-ketoglutaramate (10 mM) depressed the animals' nocturnal locomotor activity, and at higher doses induced circling behavior and myoclonus. The concentration of α-ketoglutaramate in cerebrospinal fluid appears to be a reliable diagnostic indicator of hepatic coma, and its accumulation may contribute to the pathogenesis of this disease.*

Ammonia is believed to play a role in the pathogenesis of hepatic coma, since hyperammonemia is a prominent finding in patients with this condition, and the currently most effective therapeutic measures are directed toward reducing the plasma ammonia concentrations (*1*). Also, a neuropathological picture resembling that of human hepatic encephalopathy can be induced in monkeys after chronic ammonia infusions (*2*). However, a causative relation between the elevated ammonia concentrations in plasma and cerebrospinal fluid (CSF) and the encephalopathy has not been firmly established. A major difficulty in this regard is the observation that patients with liver disease and prominent neurological symptoms can exhibit normal or only moderately elevated concentrations of ammonia in CSF and blood (*3*).

Bessman and Bessman (*4*) suggested that the toxic effect of ammonia in brain results from depletion of α-ketoglutarate, leading to a reduction in tissue adenosine triphosphate (ATP) concentrations. Subsequent studies (*5*) have failed to substantiate this proposal.

Warren and Schenker (*6*) reported that methionine sulfoximine, an inhibitor of glutamine synthetase, protected mice against acute ammonia intoxication, although, paradoxically, ammonia concentrations in brain more than doubled. As this effect is apparently not secondary to energy sparing (*7*), it could conceivably result from the inhibition of the synthesis of a toxic agent formed after the glutamine synthetase step. Glutamine, itself, exhibits no known neurotoxicity (*8*). α-Ketoglutaramate (α-KGM), a metabolite of glutamine formed by transamination with an appropriate α-keto acid (*9*),



has not been explored in this context although glutamine transaminase and ω-amidase are present in brain (*10*). On the hypothesis that α-KGM (or its cyclic isomer 2-hydroxy-5-oxoproline) might be involved in the pathogenesis of hepatic coma, we measured its concentration in the cerebrospinal fluids of human subjects, and assessed its pharmacology by perfusing it into the lateral cerebral ventricles of free-running rats.

Patients diagnosed with (i) hepatic coma, (ii) demonstrated liver disease unaccompanied by neurological symptoms, (iii) respiratory failure and obtundation, and (iv) various neurological diseases but without liver impairment, were studied. CSF was obtained from routine diagnostic lumbar puncture and was immediately frozen at − 80°C. The samples were deproteinized with 3*M* perchloric acid and neu-

Table 1. Concentrations of ammonia, glutamate, glutamine, and α-ketoglutaramate in cerebrospinal fluid of human subjects. The data, expressed as micromoles per liter of CSF, are the mean ± S.D. for the number of patients shown in brackets. The range of individual values is given in parentheses.

Condition	Concentrations (μmole/liter)			
	Ammonia	Glutamate	Glutamine	α-Ketoglutaramate
Nonhepatic controls [9]	67.7 ± 45.6 (21.0–134)	3.6 ± 2.9* (< 1.0–9.0)	422 ± 46.6 (329–477)	3.6 ± 3.2* (< 1.0–8.7)
Respiratory failure with obtundation [2]	28.8, 60.4	6.9, 4.2	1750, 1594	< 1.0, < 1.0
Liver disease without coma [5]	37.9 ± 21.1 (13.5–56.2)	2.4 ± 1.4* (< 1.0–4.8)	576 ± 226 (350–865)	2.6 ± 2.3* (< 1.0–6.2)
Hepatic coma [8]	154.6 ± 61.8† (70.3–233)	7.6 ± 4.2‡ (2.0–14.4)	2056 ± 679§ (1030–2850)	49.0 ± 21.8§ (30.9–95.4)

* Concentrations less than 1 μ*M* were arbitrarily assigned the value of 1.0 μ*M* for statistical purposes. † $P < .01$. ‡ The difference from the nonhepatic control value is significant with $P < .05$. § $P < .001$.