rocal excitatory electrical connections between, the two ipsilateral interneurons. This scheme provides for feed-forward summation of interneuronal actions upon the common follower cell population. Shared inputs to ipsilateral pairs of interneurons are of the same sign, permitting a cascading of activity leading to amplified synaptic output from the interneuron pair. Because each interneuron mediates the same synaptic action upon a given follower cell as does its ipsilateral partner, all follower cells participate in receiving summated output. As a consequence of this arrangement, an apparent redundancy of interconnections is revealed to be capable of summation, permitting more effective synaptic action than could be provided by either a single pathway, or two totally independent parallel channels.

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4. The interneurons mediate two types of depolarizing synaptic potentials to different follower cells. The depolarizing synaptic potentials to some as yet poorly identified cells (BL_{1a} and BR_{1a}) are purely excitatory chemical PSP's. In these cells, ACh also elicits a purely excitatory response. By contrast, the depolarizing synaptic potential in other cells (BL_2 and BR_2) consists of two components: an early excitatory component and a late inhibitory component. At the resting level of membrane potential, the PSP and the ACh response are primarily depolarizing. However, at depolarized membrane potentials, the interneuron or ACh pulses produce biphasic depolarizing-hyperpolarizing responses. The properties of this dual PSP will be described in a later paper (D. Gardner and E. R. Kandel, in preparation).
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C1 Inhibitor: Evidence for Decreased Hepatic Synthesis in Hereditary Angioneurotic Edema

Abstract. *Although the $C\bar{1}$ inhibitor was detected in 5 to 10 percent of normal hepatic parenchymal cells by means of the immunofluorescent technique, none was seen in liver biopsies from two individuals with hereditary angioneurotic edema having low concentrations of $C\bar{1}$ inhibitor in the serum. In contrast, the percentages of cells which reacted with fluorescent antiserums to C4 and transferrin were normal. These data suggest that in most subjects with hereditary angioneurotic edema, there is decreased synthesis of the $C\bar{1}$ inhibitor but normal synthesis of C4, and that the disease results from this biosynthetic error.*

Hereditary angioneurotic edema (HANE) is inherited as an autosomal dominant trait. Afflicted individuals tend to sustain recurrent episodes of circumscribed noninflammatory edema of the skin and the gastrointestinal and respiratory tracts (*1*). All patients with the disease are deficient in serum inhibition of the activated first component of complement, $C\bar{1}$ (*2*). The $C\bar{1}$ inhibitor of normal plasma has been isolated (*3*); it inhibits plasmin, PF/dil, and kallikrein as well as $C\bar{1}$ (*4*). In approximately 85 percent of affected kindred, the concentration of $C\bar{1}$ inhibitor protein is low, ranging from 5 to 30 percent of the normal amount as judged by immunochemical methods. In the remaining families there is a dysfunctional protein, the concentration of which is normal or elevated (*5*). The electrophoretic mobility of the dysfunctional protein varies from family to family; therefore there are probably several different aberrant structural genes producing this form of the disease (*6*).

In affected individuals, serum concentrations of the fourth and second components of complement (C4 and C2) may be low between attacks and decline during attacks (*7*). The decreased concentration of C4 is primarily related to increased catabolism, although synthetic rates may be low (see *8*).

It is not known whether the low concentrations of $C\bar{1}$ inhibitor in serums of patients with the common form of HANE are the result of diminished synthesis or accelerated destruction of the molecule. We have examined this question through the use of fluorescein-labeled antibody to localize $C\bar{1}$ inhibitor in normal liver and in liver of two patients with HANE and low serum concentrations of $C\bar{1}$ inhibitor. Biopsies of liver, duodenum, stomach, and lymph node were obtained from a 54-year-old male patient with HANE (W.K.) during hemigastrectomy for an obstructing duodenal ulcer. Liver biopsy from another patient, a 39-year-old female (G.G.), was obtained dur-

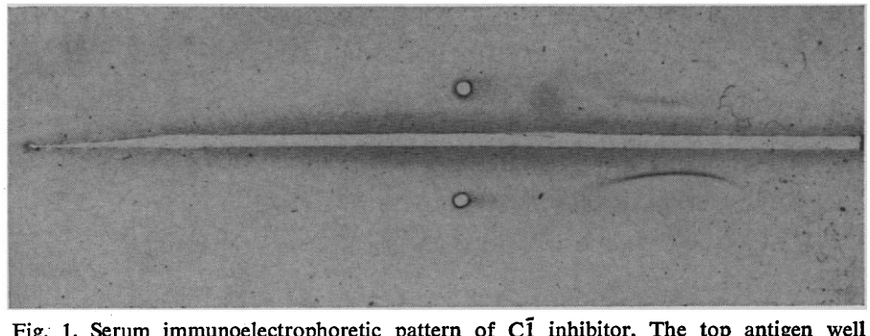

Fig. 1. Serum immunoelectrophoretic pattern of $C\bar{1}$ inhibitor. The top antigen well contained serum from a patient with hereditary angioneurotic edema (G.G.); the bottom well contained pooled serum from normal adults. The pattern was developed with goat antiserum to human α_2-neuraminoglycoprotein ($C\bar{1}$ inhibitor). A single immunoprecipitin arc is present for each sample.

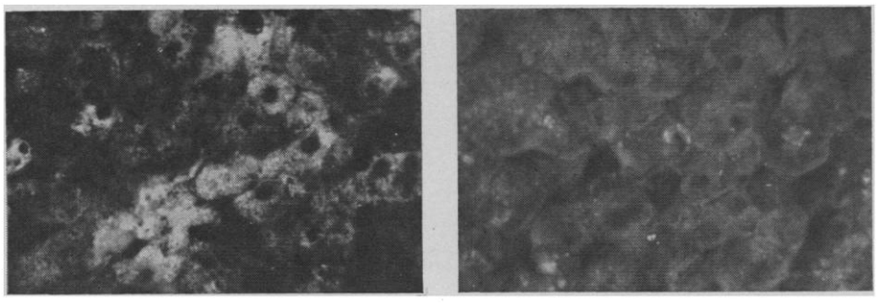

Fig. 2. Immunofluorescent staining for $C\bar{1}$ inhibitor of normal liver (left) and liver from a patient (W.K.) with hereditary angioneurotic edema (right). Sections fixed in ethanol were treated with goat antiserum to human α_2-neuraminoglycoprotein. After being thoroughly washed, they were treated with fluorescein-labeled guinea pig antiserum to goat IgG. Specific fluorescence is noted only in the cytoplasm of normal parenchymal cells.

ing hysterectomy for leiomyomata. Serum concentrations of $C\bar{1}$ inhibitor were 8 percent (1.5 mg/100 ml) and 5 percent (0.9 mg/100 ml) of normal, respectively.

Tissues were fixed in cold 95 percent ethanol with 1 percent acetic acid for 4 to 18 hours or in 3 percent glutaraldehyde buffered with neutral 0.15*M* phosphate for 1 hour before being processed for immunofluorescent studies as described by Sainte-Marie (*9*). Monospecific antiserum to $C\bar{1}$ inhibitor was elicited in goats treated with highly purified α_2-neuraminoglycoprotein (*10*), which is antigenically identical to C1 inhibitor (*11*). This antiserum gave a single strong precipitin arc with normal human serum and a barely detectable arc with the patients' serums (Fig. 1). As controls, monospecific antiserums to highly purified human transferrin and C4 (*12*) were also used. Tissue sections were incubated first with either goat antiserum to α_2-neuraminoglycoprotein ($C\bar{1}$ inhibitor), goat antiserum to transferrin, or rabbit antiserum to C4. They were then washed thoroughly with phosphate-buffered saline (*p*H 7.4) and incubated with fluorescein-labeled immunoglobulin G (IgG) from guinea pig antiserum to goat or rabbit IgG (*13*). After being thoroughly washed, the sections were examined for specific fluorescence. Controls consisted of (i) substitution of nonimmune serum (dog, goat, or rabbit), saline, or horse antiserum to whole (unfractionated) human serum for the first antiserum; (ii) absorption of the first antiserum with whole human serum or specific antigen (*10, 12*); (iii) substitution of fluorescein-labeled guinea pig antiserum to an antigen other than the first antiserum; and (iv) omission of either antiserum. No specific fluorescence was noted in any of the controls.

Specific fluorescence of $C\bar{1}$ inhibitor in the cytoplasm of individual parenchymal liver cells was seen in sections of liver from three normal subjects (Fig. 2A). In one instance 6.8 percent (of 1385 cells) and in another 7.5 percent (of 2015 cells) contained $C\bar{1}$ inhibitor. In the third, between 5 and 10 percent of the parenchymal cells showed fluorescence, although lack of resolution prevented an accurate cell count. No specific fluorescence of cells other than parenchymal cells was noted. No liver cells from the deficient patients contained detectable $C\bar{1}$ inhibitor fluorescence (Fig. 2B) despite a diligent survey of at least 100,000 cells per patient. The failure to find even minimum specific fluorescence in the patients' liver cells probably resulted from the limitations of the immunohistochemical technique. No specific fluorescence was detected in sections of W.K.'s duodenum, stomach, or lymph nodes. By contrast, sections of both normal livers and patients' livers reacted with antiserums to transferrin and C4 showed specific cytoplasmic fluorescence in individual parenchymal cells, as reported previously by Lane for rat transferrin (*14*). The percentages of fluorescent parenchymal cells when treated with antiserum to C4 were 1.9 and 4.4 for normal controls and 2.8 and 5.8 for patients.

Slices of fetal liver will incorporate radiolabeled amino acids into $C\bar{1}$ inhibitor, as demonstrated by radioimmunoelectrophoresis (*15*). However, some incorporation is found with other reticuloendothelial tissues and with fibroblasts. The absence of specific $C\bar{1}$ inhibitor fluorescence in Kupffer cells or connective tissue suggests that the hepatic parenchymal cells, rather than the reticuloendothelial system or fibroblasts, are probably the primary site of synthesis of this protein. Similarly, it is likely that the parenchymal cells synthesize C4 in vivo. With the latter protein, however, some fluorescence was also noted in Kupffer cells.

These studies suggest that in patients with hereditary angioneurotic edema and low concentrations of immunoreactive $C\bar{1}$ inhibitor there may be deficient hepatic synthesis of the protein, and that the disease results from this biosynthetic error.

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