tive correlation between the amount of Sr^{85} in the urine and that in the stool, a large amount excreted in the urine reducing the body burden and leaving less to be excreted in the stool.

To calculate the initial body burden of Sr^{90}, total fecal excretion for 12 days was divided by 0.85 on the assumption that net absorption was 15 percent (*7, 8*). Actual absorption varies somewhat, but the errors in using this factor are relatively minor.

From the data given by Bradley *et al.* (*4*), we can arrive at estimates of body burden that agree with theirs. However, much of this agreement is fortuitous. Because of the variability in the relation between urinary calcium and urinary strontium, the true values may differ by roughly 50 percent from the values found, and in some cases by even more. This variability creates difficulties in all methods used for calculating body retention from assays of urinary Sr^{90}. Calculation from the amount of Sr^{90} in the plasma does not avoid the difficulty, as the rate at which the concentration decreases in the plasma also varies from one patient to another. Although these objections do not apply to the estimation of Sr^{90} body content from secondary x-rays, this method presents other difficulties. The method reported here, therefore, has two major advantages: first, its relative simplicity, and second, its taking into account the relation between the amounts of calcium and radiostrontium excreted in the urine. For many purposes the results obtained are sufficiently accurate and can be practically useful.

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Hereditary Angioneurotic Edema: Two Genetic Variants

Abstract. *Serums of patients with hereditary angioneurotic edema lack inhibitory activity against the esterase derived from the first component of complement. In one group of patients this lack appears to result from failure to synthesize the esterase inhibitor of the first component of complement, whereas in another group of patients an abnormal, nonfunctional protein is synthesized.*

The serums of patients with hereditary angioneurotic edema (HANE) are unique in that they do not inhibit the hydrolysis of *N*-acetyl-L-tyrosine ethyl ester by the esterase derived from the first component of complement (C'1) (*1*). This observation led to the conclusion that serums of such patients lack the C'1 esterase inhibitor (EI). Further support for this observation was obtained from the demonstration that the titer of the fourth component of complement is decreased in vivo in the blood of these patients and that the free esterolytic activity in their serum or plasma has similar ultracentrifugal characteristics to C'1 esterase (*1*).

The C'1 esterase inhibitor has been isolated in a highly purified state; it is an acid labile α_2 globulin (*2*). In order to estimate the amount of this inhibitor in normal serums and in the serums of patients with hereditary angioneurotic edema by immunochemical means, two rabbits were repeatedly injected in the foot pads during the course of 1 year with a total of 4 mg of the purified inhibitor in complete adjuvant. Upon immunoelectrophoresis, the rabbit antiserum formed three bands of precipitation with normal human serum. This antiserum could be made specific for the inhibitor without diminution in its potency, and two contaminating bands could be eliminated by mixing 20 parts of the rabbit antiserum with 1 part of the serum from a patient whose serum contained only trace amounts of the inhibitor. The antiserum was titrated by agar-gel diffusion (*3*) against a highly purified preparation of the inhibitor freed of contaminating protein as measured by immunologic criteria. The concentration of the inhibitor in normal serum was 2.4 ± 0.4 mg per 100 ml.

The serums of 25 patients from ten kindreds with hereditary angioneurotic edema contained 0.16 to 0.64 mg of EI per 100 ml. Upon immunoelectrophoresis, these 25 serums formed only faint bands of precipitation with antiserum against EI. The serums of the "normal" relatives of the 25 patients contained normal concentrations of the inhibitor. In contrast, the serums of nine patients in two additional affected kindreds contained normal amounts of inhibitor as estimated by immunochemical means, even though these serums had no inhibitory activity in the esterolytic assay. The rabbit antibody to the inhibitor prepared from a pool of normal human serums gave a reaction of complete identity with the nonfunctional inhibitor of these nine patients, an indication that the abnormal protein was not deficient in antigenic determinants (Fig. 1). Upon immunoelectrophoretic analysis in barbital buffer at *p*H 8.2 (ionic-strength 0.05), no difference could be discerned between the bands of precipitation formed with EI in normal serums or with the nonfunctional inhibitor in the serums of patients. However, immunoelectrophoresis in the presence of calcium lactate, as outlined by Hirschfeld (*4*), showed a clear differ-

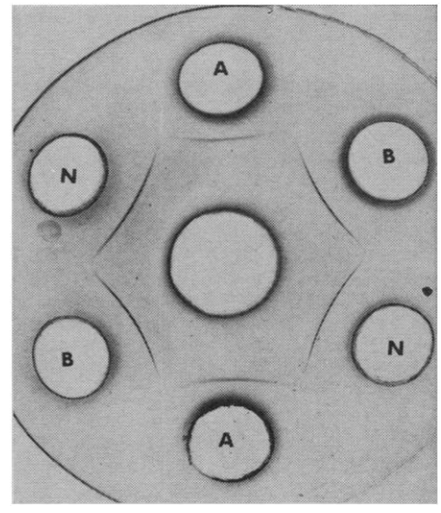

Fig. 1. Agar double diffusion of serum from normal humans (*N*), serum deficient in EI from patients with hereditary angioneurotic edema (*A*), and serum from patients synthesizing nonfunctional EI (*B*) against rabbit antiserum to EI.

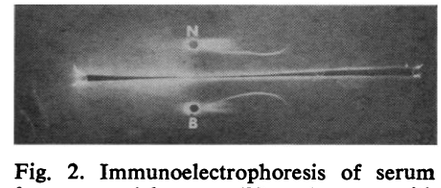

Fig. 2. Immunoelectrophoresis of serum from normal humans (*N*) and serum with nonfunctional EI from a patient with hereditary angioneurotic edema (*B*) against rabbit antiserum to EI. The anode is to the right.

ence between the normal and abnormal serums in that a characteristic double-arc "gull-wing" band of precipitation was formed by the inhibitor in normal serum, whereas that in the abnormal serum formed only a single component which corresponded to the fast portion of the normal "gull-wing" pattern (Fig. 2). These differences could not be attributed to the effect of concentration or *p*H and were believed to result from a structural alteration in the nonfunctional protein. Inasmuch as the reaction of identity was found on Ouchterlony analysis, the altered immunoelectrophoretic pattern implies a structural alteration in the nonfunctional inhibitor without deleting antigenic determinants.

Hereditary angioneurotic edema is transmitted as an autosomal dominant characteristic, and studies of pedigrees indicate that patients with the disease are heterozygotes. However, the serums of patients in 10 of the 12 kindreds studied contained only 6 to 25 percent of the normal amount of inhibitor. The deficiency of antigenic inhibitor in these serums results from a defect in synthesis of EI since a study of I^{131}-labeled inhibitor showed that the rate of catabolism of the inhibitor in the patients did not differ from that in the normal subjects (*5*). In the two other kindreds a normal amount of nonfunctional, structurally altered inhibitor is synthesized. The two genetic variants of hereditary angioneurotic edema may be analagous to the two types of tryptophane synthetase mutants of *Neurospora crassa*: one type lacks the enzyme, and, in the other type, enzymatically inactive protein is synthesized and this inactive protein is antigenically cross-reacting (*6*). Possibly synthesis by a heterozygous individual of a nonfunctional, abnormal protein might suppress the regulatory mechanism for the synthesis of normal, functional protein (*7*). Further study of the genetic variant in which a nonfunctional protein is synthesized may confirm the hypothesis.

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Photoreception and Entrainment of Cockroach Activity Rhythms

Abstract. *In the cockroach there are two distinct sets of photoreceptors—the compound eyes and the ocelli—which may function as a sensory input whereby circadian locomotor rhythms are entrained by environmental light cycles. Surgical removal of the ocelli did not interfere with normal entrainment, but covering over the compound eyes effected a loss of entrainment.*

One of the striking features of the circadian rhythmic system is that it can be entrained by light cycles. The word circadian (*circadiem*, about a day) has been widely adopted to emphasize the fact that under constant conditions with respect to light and temperature the periodicity of rhythms in plants and animals, although nearly equal to 24 hours, may be consistently greater or less than this value. For example, the locomotor rhythm of a given animal might show an average period (interval between successive daily onsets of activity) of 23.5 hours instead of 24.0 hours. Such a rhythm is considered to be a "free-running" rhythm—that is, it is not being entained to the frequency of a natural environmental cycle. However, the period of such a rhythm may be entrained to precisely 24.0 hours by regular cycles of light and darkness, for example, 12 hours of light alternating with 12 hours of dark (LD 12 :12). One aspect of this entrainment phenomenon is the question of the site of the sensory input which channels the information about environmental light to the endogenous circadian system. In metazoans with distinct photoreceptors, such as eyes or ocelli, it has generally been assumed that one or more of these act to relay information about the frequency of the light cycle to the circadian system. Recent studies on insects suggest that particular caution should be used in making such an interpretation because, first, there are conflicting statements in the literature and, second, Lees (*1*) found that photoperiodic control of reproduction in aphids is mediated by light falling directly on the pars-intercerebralis of the brain. This latter observation is particularly significant in view of the evidence that circadian rhythms are involved in photoperiodic time-measurement (*2*).

The studies described herein were conducted with the cockroaches *Periplaneta americana* and *Leucophaea maderae*. These insects have two pairs of external photoreceptors: the large compound eyes and a pair of much smaller ocelli lying adjacent to the antennae sockets. Investigators have generally agreed (*3*) that the ocelli augment photokinetic reactions normally mediated by the compound eyes. A wholly new function is explicit in the claim by Harker (*4*) that the locomotor rhythm in the roach, *Periplaneta americana*, is synchronized to LD cycles through the ocelli and not the compound eyes. It should be noted that Harker's results contradict the earlier findings of Cloudsley-Thompson (*5*) which implied, although no supporting data were given, that both sets of receptors must be intact to maintain a synchronized rhythm in *Periplaneta*. More recent experiments on the house cricket, *Gryllus domesticus*, by Nowosielski and Patton (*6*) suggest that entrainment can be mediated by either pair of photoreceptors. In one major respect the results of our studies with roaches lead us to disagree with the earlier reports; namely, we suggest that the sensory input to the circadian system is either through the compound eyes or possibly by direct photostimulation of the brain. Although the evidence does not yet permit a definite choice between these alternatives, it does clearly rule out the involvement of the ocelli.

Our conclusions are based on experiments in which we painted over the photoreceptors, or in some cases removed the ocelli by surgery. After testing various "paint" materials we