macromolecules which might be related to the specific antibodies produced in response to immunization (*5*). It remains to be determined whether the differences between his observations and ours are related to the species of animal, the route of immunization, or the techniques for studying macromolecular uptake.

W. ALLAN WALKER
KURT J. ISSELBACHER
Gastrointestinal Unit, Massachusetts General Hospital, Boston 02114
KURT J. BLOCH
Arthritis and Clinical Immunology Units, Massachusetts General Hospital

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Deficiency of Kallikrein Activity in Plasma of Patients with Cystic Fibrosis

Abstract. *Total kallikrein activity and kallikrein activity inhibited by soybean trypsin inhibitor are significantly reduced in the plasma of patients with cystic fibrosis compared to age-matched controls. The level of the STI inhibited kallikrein activity in the plasma of heterozygotes was significantly different from that in either controls or affected children. However, the individual heterozygote could not be reliably identified in each case.*

Cystic fibrosis (CF) is a familial metabolic disorder in which the basic defect is unknown (*1*). Several studies have indicated that saliva and serum of patients with cystic fibrosis contain "factors" which may be unique to this disease (*2–4*). Saliva of patients with cystic fibrosis has been reported to contain a macromolecular factor which inhibits sodium reabsorption (*2*), while serum of these patients contains factors which induce dyskinesis in the rhythmic beat of cilia from rabbit trachea (*3*) and from oysters (*4*).

Earlier studies in our laboratory, undertaken to explain the presence of the macromolecular factor in saliva of patients with cystic fibrosis, indicated that trypsinlike activity was deficient in the saliva of these patients (*5*). The trypsinlike activity present in normal saliva was similar in a number of its properties to those of kallikreins. Since kallikreins are present in human plasma (*6*), we have extended our studies to include the determination of kallikrein activities in plasma of controls and patients with cystic fibrosis. The results of these studies show that plasma of patients with cystic fibrosis is deficient in kallikrein activity as compared to that of either age-matched or adult controls.

We have assayed kallikrein activity as arginine esterase activated by treating plasma with chloroform and ellagic acid. This assay is valid in view of the findings of Colman *et al.* (*6*), who have shown that the level of arginine esterase activity is proportional to the concentration of Hageman factor activated by ellagic acid. Since Hageman factor is a physiologic activator of prekallikrein, we, like Colman *et al.* (*6*), assume that the arginine esterase activity reported below represents kallikrein activity.

Blood was collected in citrated plastic tubes, and contact with glass surfaces was avoided. The plasma was separated by centrifugation and either used immediately or stored at −20°C. The plasma, in siliconized tubes, was treated with an equal volume of cold chloroform and centrifuged at 4°C. The kallikrein in the plasma fraction was activated by treating with ellagic acid (final concentration 0.05 m*M*) for 15 minutes at 25°C. A portion (0.3 ml) of this plasma was transferred to tubes containing 0.015*M* α-*N*-(*p*-toluenesulfonyl)-L-arginine methyl ester (TAME), 0.10*M* phosphate buffer (*p*H 7.6), and 0.15*M* NaCl. The reaction mixture [final volume, 1.0 ml (*6*)] was incubated at 37°C for 15 minutes, and the reaction was terminated by the addition of 1.0 ml of 10 percent trichloroacetic acid. The mixture was centrifuged and the methanol in the supernatant was assayed according to Siegelman *et al.* (*7*). Activity was assayed in the presence and absence of soybean trypsin inhibitor (STI).

Plasma treated with chloroform and ellagic acid contains at least two types of activities. One type of activity is inhibited by low concentrations of STI (5 μg/ml), whereas the other is resistant even to very high concentrations of inhibitor. In order to assay for both

Table 1. Kallikrein activity in plasma. The results are expressed as micromoles of TAME utilized per hour per milliliter of plasma.

Sample	*N*	Total activity		STI-inhibited activity		STI-resistant activity	
		M ± S.D.	Range	M ± S.D.	Range	M ± S.D.	Range
Normal adults	15	43.3 ± 17.7	20.2–73.5	30.2 ± 15.3	12.9–69.1	13.1 ± 13.1	1.1–51.6
Normal children	20	41.2 ± 7.6	30.5–55.1	23.2 ± 6.2	14.1–35.6	17.9 ± 5.4	10.1–31.4
Cystic fibrosis (CF_1)	20	16.8 ± 6.9	2.4–27.6	9.6 ± 4.2	0.5–17.6	7.2 ± 4.0	1.9–17.2
Cystic fibrosis (CF_2)	17	26.6 ± 12.3	9.9–50.4	13.0 ± 6.9	3.4–29.5	13.3 ± 8.7	3.0–32.5
Parents	27	32.5 ± 10.4	14.2–64.0	18.4 ± 8.4	6.1–47.2	14.8 ± 8.8	1.8–30.0
All controls	35	42.1 ± 13.0	20.2–73.5	26.2 ± 11.6	12.9–69.1	15.9 ± 9.8	1.1–51.6
All cystic fibrosis	37	21.3 ± 10.9	2.4–50.4	11.2 ± 5.9	0.5–29.5	10.0 ± 7.2	1.9–32.5

activities, we routinely assayed one set at an STI concentration of 200 μg/ml (6). This high concentration was utilized to ensure complete saturation of the fraction of kallikrein sensitive to the inhibitor.

Kallikrein activities in the plasma of children with cystic fibrosis (CF_1), control age-matched children, and control adults are shown in Table 1. Kallikrein activities in plasma of an additional group of patients with cystic fibrosis (CF_2), and their parents (obligate heterozygotes) are also shown in Table 1.

These data clearly demonstrate that there are significant differences between the levels of total kallikrein and STI-inhibited kallikrein activities in the plasma of control individuals and patients with cystic fibrosis ($P < .001$). The STI-inhibited component in heterozygotes was significantly different from that in their children (CF_2 being $P < .02$) and normal adults ($P < .05$).

Mixing plasma from controls and patients with cystic fibrosis either before or after activation yielded the expected intermediate levels of kallikrein activity; this result thus excluded the possibility that an inhibitor was present or that factors needed to activate prekallikrein in cystic fibrosis plasma were absent. Plasma, from controls and patients with cystic fibrosis, activated with chloroformellagic acid did not hydrolyze α-*N*-benzoyl-DL-arginine-*p*-nitroanilide, thus excluding the presence of trypsin. Initial experiments showed that levels of carboxypeptidase N and kallikrein inhibitor, two other components of the kinin system, are not significantly different in the plasma of controls compared to that in patients with cystic fibrosis. These findings indicate that the observed deficiency appears to be restricted to kallikreins in the kallikrein-kinin system. Since plasma contains a number of kallikreins (6), the reduction in total kallikrein and STI-inhibited kallikrein activities in plasma of patients with cystic fibrosis may be due to the reduction of a specific component.

Although our data show that the level of STI-inhibited component of kallikrein in the plasma of heterozygotes is significantly different from that in either controls or affected children, the individual heterozygote could not be identified in each case. In most families, parents had levels of STI-inhibited kallikrein activity intermediate between that of their children with cystic fibrosis and control adults. In two families, both the parents and the children with cystic fibrosis had levels of activity considerably higher that that of their respective groups. The reasons for the high enzyme activity in these families is not clear; however, they may represent examples of genetic heterogeneity.

The relation of the kallikrein system to the clinical manifestations of cystic fibrosis is not clear. With respect to the kallikreins, there is very little information on the effect of infection, respiratory acidosis, and other clinical manifestations of the disease. However, the reduced activity in heterozygotes suggests that the reduction in patients is probably not directly related to the clinical manifestations. It has been suggested that the adrenal glands may be overstimulated in patients with cystic fibrosis (8, 9) because of the finding of increased catecholamines in the adrenal medullas (8) and in urines of patients with cystic fibrosis (9). In experiments with healthy human volunteers, injection of high concentrations of epinephrine resulted in the reduction of kininogen and kinin (10). Such studies could possibly explain the reduction of kallikrein activity in cystic fibrosis.

Kallikreins are present in pancreas, salivary glands, plasma, sweat, and saliva (11)—organs, tissues, and secretions in which clinical and biochemical abnormalities are found in patients with cystic fibrosis (1). Therefore, the demonstration of a generalized deficiency of kallikreins may explain the clinical and biochemical manifestations of cystic fibrosis.

G. J. S. Rao, L. A. Posner
H. L. Nadler
Division of Genetics, Children's Memorial Hospital, Department of Pediatrics, Northwestern University Medical School, Chicago, Illinois 60614

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Fission in the Evolution of a Lizard Karyotype

Abstract. *The lizard* Anolis monticola *has a diploid chromosome number of 48 (24 macrochromosomes and 24 microchromosomes). More primitive members of the genus, as determined by bone morphology, have 12 macrochromosomes and 24 microchromosomes. Since the higher chromosome number is the derived condition, this is a case of karyotypic change by centric fission.*

Chromosome fusion and fission (Robertsonian change) are two alternative explanations for the relation of karyotypes that differ in diploid number but agree in the number of chromosome arms [fundamental number (1)]. Controversy over the mechanism for fission and its frequency have caused many cytogeneticists to favor fusion, often to the complete neglect of fission. Recent studies have removed the grounds for disputing the simplest possible mechanism for fission, simple splitting of the centromere. Light and electron microscopy have shown that the centromere of a bi-armed chromosome contains twice the material in the centromere of a telocentric chromosome (2). Stable telocentric chromosomes, including some that are almost certainly fission products (3), have been demonstrated (2, 4). The frequency and importance of fission in karyotype evolution, however, remain undetermined (5). Cases are needed in which the direction of Robertsonian change can be demonstrated by unequivocal phylogenetic evidence. We present such a case here.

Phyletic relationships among West Indian species of *Anolis*, a large Neotropical genus of iguanid lizards, are now well worked out (6). There is karyotypic information for approxi-