the organism. The number of such loci does not have to be large for this probability to be so small that the event can be, for practical purposes, considered never to have occurred.

The alternative to an abrupt code change is a gradual one. However, because part of the code is expressed as independent codons throughout the genome there is no way in which an organism could gradually change from one code to another without passing through a random phase in which more than one amino acid can be placed in a locus. It is hard to imagine any circumstance under which a selective advantage would be gained by the random placement of certain protein amino acids. In fact, a change of this kind would almost certainly have large scale deleterious effects on any organism and therefore the change would not be perpetuated. Thus, once established, the genetic code will never change, barring an incredible event, and all organisms descendant from a given organism having the complete code will all have the same code.

We have postulated that the C site on the adaptor molecule cannot change permanently because it must match large numbers of codons. The A site of the aa-enzyme is also restricted because it must match, possibly with varying efficiency, amino acids which are invariant in structure. Therefore, site A (and its amino acid) and C remain permanently associated as if their relationship were chemically determined. The R site of the aa-enzyme and the E site of the adaptor molecule are, however, not restricted in this sense, since as long as the two sites change together the system will operate. Thus a mutation taking place in the R site may slightly change its fit to the existing E site. A subsequent mutation in the E site may then restore the previous mutual relationship. In this manner the genetic code remains invariant while the R and E sites change as if in unison. Differences in the R and E sites in different species would therefore be expected, and in fact are found (*3*). It is also possible that one aa-enzyme could attach a given amino acid to more than one of the aRNAs coding for that amino acid. In other words, in degenerate situations one aa-enzyme could attach a given amino acid to several different aRNAs coding for that amino acid regardless of the nature of the C site.

Because the genetic code should remain invariant, its constancy can be used to establish the number of primordial ancestors from which all (present) organisms are derived. If, for example, the code is universal, and there is evidence that it might be (*7*), then all existing organisms would be descendants of a single organism or species. If the code is not universal, the number of different codes should represent the number of different primordial ancestors that either existed during the time the present code was being completed, or existed when organisms were so simple that changes in practically all proteins were not always fatal. In either case, if different codes do exist they should be associated with major taxonomic groups such as phyla or kingdoms that have their roots far in the past.

An alternative explanation for a universal code is that the specific association between a given amino acid and its codon is determined by chemical phenomena such as those, for example, which lead to the association in a dipolar form of two atoms of hydrogen and one of oxygen to form a molecule of water. Clearly the structure of water need not be genetically prescribed. Although a chemically determined code cannot be ruled out there is little evidence for it.

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17 September 1963

Antibacterial and Enzymic Basic Proteins from Leukocyte Lysosomes: Separation and Identification

Abstract. *A lysosomal fraction from polymorphonuclear leukocytes has been directly analyzed for basic proteins by zone electrophoresis. Evidence for the presence of bactericidal basic proteins in polymorphonuclear leukocyte lysosomes is presented.*

Over the past decades various workers have reported bactericidal activity in extracts of polymorphonuclear leukocytes (PMN) (*1*). As a result of de Duve's description of mammalian cell lysosomes, attention has been drawn towards the study of PMN granules, which disappear during phagocytosis. Cohn and Hirsch (*2*) have reported, in rabbit PMN granules, the localization of several hydrolytic enzymes including ribonuclease, deoxyribonuclease, and lysozyme (which are basic or cationic, proteins) as well as the bulk of a bactericidal principle, "phagocytin." Using histochemical techniques, Spitznagel and Chi (*3*) have presented evidence that the antibacterial action of PMN during phagocytosis and degranulation may be due to transfer of basic proteins from PMN lysosomes to ingested bacterial cells. Purification and separation of the antibacterial substances from the enzymic basic proteins of PMN lysosomes has not yet been achieved; hence the precise physical and chemical properties, and indeed the mode of antibacterial action of substances from the PMN lysosomes, remain to be defined.

Using zone electrophoresis we have resolved the PMN lysosomes into several components with recognizable biological and biochemical properties. The components studied include three which possess both bactericidal activity and the electrophoretic mobilities of highly basic proteins.

A lysosomal fraction was prepared from guinea-pig peritoneal exudates induced by injection of 0.5 percent

glycogen in 0.85 percent saline; the exudates contained 95 percent PMN. The cells were homogenized and subjected to differential centrifugation in 0.25*M* sucrose at 4°C. A sample of the lysosomal suspension (8000*g* fraction) in 0.25*M* sucrose was applied to a strip of Oxoid paper (20 × 5 cm). Prior to the application the paper was soaked in 0.5 percent cetyltrimethyl ammonium bromide (CTAB) for 3 minutes and rinsed with acetate buffer, *p*H 4, for 5 minutes. Electrophoresis (200 v, 0.002 amp) was carried out for 1 hour at room temperature in acetate buffer, *p*H 4, 0.05 ionic strength. A part of the strip stained with amido black was used as a marker for eluting the individual bands for the assay of biological activity. For antibacterial assay, protein was eluted from the bands and dialyzed against 0.01*N* HCl for 6 hours to remove the residual CTAB. The assay was carried out in 2-ml plastic cups containing twofold dilutions of eluate in citrate-phosphate buffer, *p*H 5.6, to which were added 200 to 250 bacteria from an 18-hour washed culture of *Escherichia coli* (*4*). After the cups were incubated for 2 hours at 37°C, melted nutrient agar, in which triphenyltetrazolium chloride had been incorporated to facilitate early visualization of colonies, was added to each cup. The cups were then incubated at 37°C for 18 to 24 hours. Fifty percent inhibition of growth as estimated by comparison with control cups was taken as the end point. Assays for lysozyme (*5*), ribonuclease (*6*), and deoxyribonuclease (*7*) were carried out on protein which had been eluted from each band with appropriate buffers.

The consistently reproducible electrophoretic pattern obtained from the lysosomes is shown in Fig. 1. The presence of CTAB, a cationic detergent, and the acid buffer on the Oxoid strip was instrumental in causing immediate lysis of the applied lysosomes; subsequent electrophoresis on the treated paper resolved the components into seven or more bands moving towards the cathode. A qualitatively identical pattern was obtained with lysosomes from peripherally circulating PMN. "Phagocytin" prepared from PMN lysosomes (*2*) was resolved into a similar pattern consisting of seven or more bands.

Assay of the eluted bands showed that antibacterial activity was confined to three bands which migrated most

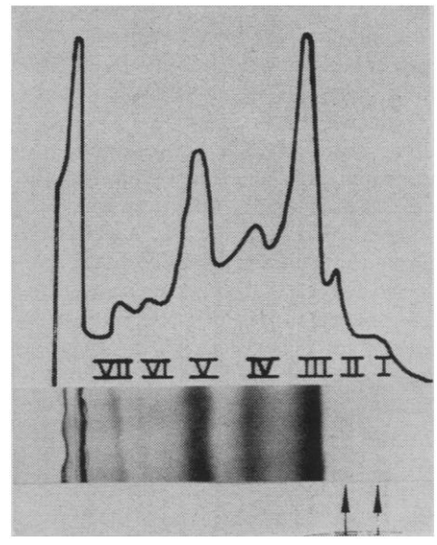

Fig. 1. Zone electrophoresis of PMN lysosomes. Cathode on the right, Migration to the right. Antibacterial bands, I, II and III; lysozyme, IV; RNAase, V; DNAase, VI. The graph represents the relative concentrations of proteins. Bands I and II, which are faint though unmistakable, are indicated by arrows.

rapidly towards the cathode (I, II, and III of Fig. 1), while lysozyme activity was found exclusively in band IV. Ribonuclease was detectable only in band V. Band VI possessed deoxyribonuclease activity (Table 1). Samples of purified commercial lysozyme, ribonuclease, and deoxyribonuclease displayed mobilities identical to bands IV, V, and VI, respectively.

In another series of experiments eluates from bands I, II, and III caused heavy agglutination of bacteria in phosphate buffer, *p*H 6.2, while the other bands did not manifest such activity. Microscopic examination of bacteria treated with material from bands I, II, and III did not show any evidence of lysis despite heavy agglutination and prolonged incubation at 37°C.

Table 1. Relative protein content and biological activities of components obtained by electrophoresis of PMN lysosomes. Band IV yielded activity equivalent to 0.63 μg of crystalline egg lysozyme. Band V yielded activity equivalent to 0.5 μg of crystalline pancreatic ribonuclease. Band VI yielded the equivalent of 0.2 μg crystalline pancreatic deoxyribonuclease.

Band	Mobility (μ)*	Relative protein content†	Antibacterial activity‡
I + II	0.65	10	16
III	.53	24	32
IV§	.40	18	0
V§	.29	20	0
VI§	.14	3	0
VII	.10	4	0
Origin	0	21	0

* μ ($cm^2hr^{-1}v^{-1}$) at *p*H 4.0. † Relative percentage of amido black per band. ‡ Reciprocal of highest dilution showing 50 percent growth inhibition. § Samples of purified commercial lysozyme, ribonuclease and deoxyribonuclease displayed mobilities in this system identical to bands IV, V, and VI, respectively.

The antibacterial components were highly reactive with acidic substances as evidenced by the formation of visible precipitates with RNA, DNA, and heparin. Highly polymerized DNA was precipitated in the form of long white strands. The antibacterial fractions showed bactericidal activity against gram-positive and gram-negative organisms and *Candida albicans*.

Fractional precipitation with ethanol, according to the method of Ui (*8*), was carried out on PMN lysosomes extracted with 0.2*N* H_2SO_4. The fraction precipitable with 20 percent ethanol accounted for most of the antibacterial activity in the lysosomes, and on electrophoresis it was resolved into bands corresponding to I, II, and III of the intact lysosomes. To organisms tested, this fraction imparted some of the histochemical characteristics of cationic particles including staining with fast green which had been adjusted to *p*H 8.1 with NaOH (*9*). The antibacterial components could be removed from the solution by absorption with the bacteria.

Amino acid analysis (*10*) of the 20 percent ethanol fraction consisting of the antibacterial bands I, II, and III showed that basic amino acids accounted for 25 percent of the total amino acids. Arginine alone represented 15 percent of the amino acids.

Our results show that the proteins in electrophoretic fractions I, II, and III are cationic in nature, possessing bactericidal activity. On the contrary, bands IV, V, and VI, which exhibit lysozyme, ribonuclease, and deoxyribonuclease activity, respectively, show no, or only traces of, antibacterial activity. The electrophoretic mobilities of bands I, II, and III seem to be sufficiently different from those of known antibacterial basic proteins in the tissues, to infer that here we probably are dealing with unique compounds. They are among the most basic proteins in the tissues since they migrate to the cathode more rapidly than even the well-known basic proteins, lysozyme, and ribonuclease. The relatively high content of arginine also tends to support this conclusion. The resolution

of other enzymes in lysosomes requires further investigation. They may remain at the origin on the Oxoid paper under the conditions studied while the basic protein enzymes migrate toward the cathode (*11*).

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6 September 1963

Running as Both a Positive and Negative Reinforcer

Abstract. *Rats were required to press a bar to activate a motor-driven wheel that forced them to run and subsequently to drink to turn off the wheel. Barpressing and licking increased, showing the onset and offset of running to be positively and negatively reinforcing, respectively. The experimental control of the offset of running, in contrast to the traditional control for onset only, served to demonstrate that since organisms stop such behaviors as they start, self-initiated behaviors will act as negative as well as positive reinforcers.*

The traditional use of two kinds of events for positive and negative reinforcement, respectively, creates the impression that the environment of a species divides naturally into discrete classes of positive and negative events. In fact, this division results more from an experimenter convention than from a relation between the species and its environment. Specifically, it results from the fact that experimenters instrument only the onset of some behaviors and only the offset of others, rather than using both the onset and offset of any one behavior.

For example, although organisms both initiate and terminate eating, only initiation is used in reinforcement. In the standard food reinforcement case, the organism is required to make an arbitrary response (for example, barpress) to produce food and thus eat; but it is not required to make an additional response to turn food off and thus stop eating. On the contrary, the food delivered per reinforcement is less than the organism normally eats per burst of eating, and thus the usual disposition to terminate eating does not arise.

On the other side of the coin, only the organism's tendency to turn off (for example) electric shock is used. But will organisms initiate contact with shock and other supposedly negative events? Recent work (*1*) shows that rats initiate contact with electric shock, and fail to do so only at "high" voltages. Except for the "high-intensity" cases, organisms apparently initiate and terminate responding for all stimuli to which they respond. That is, they not only initiate the traditional positives, and terminate the traditional negatives, but rather initiate and terminate both. Indeed, all free responding is highly discontinuous, there being apparently characteristic burst length and interburst length distributions for each behavior (*2*). Accordingly, to demonstrate the positive and negative capacities of one and the same event requires that there be experimental control of both onset and offset, not one or the other as has been the case.

Of the three cases for which we are currently attempting to establish control of both onset and offset, the one reported here is locomotion. Two findings aided the implementation of this case. (i) Rats choose to press a bar that causes a wheel to rotate and force themselves to run. That is, for the rat, the opportunity to force itself to run is reinforcing; the frequency of the bar-press is increased by such a contingency. (ii) The rat is able to drink while running. These findings led to the following procedure. The rat is placed in a modified Wahmann activity wheel that contains a bar and a drinkometer (*3*). The wheel is not free to move but is connected to a variable-speed motor. When the rat presses the bar, the motor is activated, the wheel rotates, and the rat is forced to run. It must continue running until it licks the drinkometer a predetermined number of times, which turns off the motor, stops the wheel, and allows the rat to stop. The rat thus both starts and stops running, the former by the bar-press, the latter by licking.

The base measure for the bar-press is the usual number of bar-presses when the bar-press does not turn on the wheel. The base measure for licking is the duration of licking when licks do not turn the wheel off. That is, in determining the base lick rate, the bar-press turns the wheel on, so that the rat runs, but drinking does not turn the wheel off; instead, the experimenter turns the wheel off after each 5-second interval of running. The base condition was designed as a control for the possibility that running might either induce licking or interfere with and reduce it. In fact, running tends to reduce drinking: the rat drinks most when running is totally precluded (*4*). Because of this decremental relation, we used the 5-second running burst in the base condition; this value leads to a total duration of running per session (under 200 seconds) that is close to, but less than, the smallest amount of running found in any of the experimental conditions (see Fig. 1). Accordingly, increments in licking computed relative to this base err conservatively, that is, underestimate the increment.

Three female albino rats, about 180 days old, Sprague-Dawley strain, were used. They were maintained on free food and water. An additional question was answered by using a fixed-ratio schedule in conjunction with the "off" response. How does the "difficulty" of turning off a response affect the likelihood of its being turned on? All animals were trained with fixed-ratio lick requirements of 1, 3, 9, 19, and 13, in the order stated. That is, on different sessions the rat was required to complete a different, predetermined number of licks in order to turn the wheel off. On all sessions the drinking tube contained 8 percent sucrose by weight; sucrose was used to facilitate the drinking response. One bar-press always turned the wheel on. All sessions lasted 20 minutes and took place daily.

Figure 1 shows the principal results for one subject, results for the other two being the same in all essentials.