continuum. The difference between C-mice and D-mice was highly significant (Table 2) ($\chi^2 = 20.83$, 3 d.f., $P < .001$), and the difference between C-mice and B-mice was even more significant ($\chi^2 = 33.99$) (*5*). Only the difference between the two dextral strains, D and B, was not significant ($\chi^2 = 5.12$, $P < .20$), although inspection of Table 1 suggests a greater degree of dextral asymmetry in strain B than in strain D.

When sinistral strain C was mated to dextral strain D, the F_1 hybrids were predominantly dextral. When sinistral strain C was mated to dextral strain B, the majority of the F_1 hybrids were dextral. In cross C × D the hybrids differed significantly ($\chi^2 = 29.82$, $P < .001$) from their C-parent, and resembled their D-parent ($\chi^2 = 1.55$). In cross C × B the situation was analogous—the hybrids differed significantly ($\chi^2 = 34.20$) from parent C, but resembled parent B ($\chi^2 = 4.23$). Cross D × B is of interest because they are both dextral strains. If parent B were more dextral than parent D, one would expect that hybrids would resemble parent B rather than D. This was the case; the hybrids resembled parent B ($\chi^2 = 5.31$) but not their less dextral parent D ($\chi^2 = 10.39$, $P < .02$). Thus, septal artery dextrality appears to be a dominant trait. In the three strains we worked with, arterial sinistrality was recessive to dextrality.

These findings regarding dominance were confirmed by data obtained from backcross populations. If an F_1 hybrid is backcrossed to one of its parents, P, one expects one half of the backcross progeny, BX, to resemble that parent, and the other half to resemble the hybrid, that is, BX = ½ P + ½ F_1. In the case of traits displaying dominance this leads to two predictions: (i) Populations resulting from a backcross of F_1 to the dominant parent, P_D, will show little phenotypic variation and will tend to be unimodal. Such populations will resemble both the F_1 and the dominant parent population which, because of dominance, resemble each other, that is, BX = P_D = F_1. (ii) In contrast, populations resulting from a backcross to the recessive parent, P_R, will show maximum phenotypic variation. Such populations will tend to be bimodal since half of the animals will be like the recessive parent and the other half will be like F_1, and P_R and F_1 differ, that is, BX = ½ P_R + ½ F_1.

Our data bear out these two predictions. Population BX[(C × D) × D] resemble both the dominant parent D ($\chi^2 = 0.18$), and the hybrid parent F_1 (C × D) ($\chi^2 = 1.45$). Population BX [(C × B) × B] also resembled the dominant parent B ($\chi^2 = 3.95$); it differed though from its hybrid parent F_1 (C × B) ($\chi^2 = 9.16$, $P < .05$) by being even more "dextral" and unimodal—93.1 percent of all animals were R!. The situation was quite different in population BX[(C × B) × C]. As predicted, this backcross of F_1 (C × B) to the recessive parent C was clearly bimodal—43.8 percent of the animals were L! and 40.6 percent were R!. The situation was similar though not as striking in the case of population BX[(C × D) × C].

In general, dominance appears to evolve where in some way the dominant condition is of greater adaptive value than the recessive one. It is not known how septal artery dextrality is of benefit to mice as opposed to arterial sinistrality.

Phenotypic variation within an inbred strain or within an F_1 population of inbred strains points to nongenetic, "environmental" sources of variation. The three inbred strains and the three F_1 groups of this study displayed much phenotypic variation, forcing us to conclude that asymmetry, while undoubtedly influenced by heredity, is readily influenced by nongenetic factors. Bloor and associates (*3*) have arrived at a similar opinion in their genetic study of coronary anatomic patterns (accessory coronary ostia) in three inbred strains of rats. The demonstration that genetic factors play a role in the determination of coronary artery patterns in the mouse and in the rat adds support to reports of racial and familial similarities of coronary anatomy in man (*6*). The presence of an additional primary coronary artery or main branch to the septum could serve as a potential source of collateral circulation upon occlusion of a main coronary artery. The low incidence of myocardial infarction in Bantus has been explained on this basis (*3*).

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Atropinesterase and Cocainesterase of Rabbit Serum: Localization of the Enzyme Activity in Isozymes

Abstract. *Zymograms reveal a multiplicity of esterase isozymes in rabbit serum. Most of the staining activity is concentrated in a region of the gels just anodal to the albumins where six phenotypes (A, AF, F, M, P, and S) are distinguished. The atropinesterase activity is associated with phenotypes A and AF and appears to be restricted to a single isozyme, zone A. Cocainesterase activity is limited to isozyme S, a zone common to all phenotypes except M.*

It has long been known that some but not all rabbits possess in their serum an enzyme capable of hydrolyzing atropine (DL-hyoscyamine) and other tropine esters. This enzyme, commonly referred to as atropinesterase (atropine acyl-hydrolase, E.C. 3.1.1.0), has been the subject of an extensive literature (*1*, *2*) which traces to a report in 1852 that rabbits can thrive on a diet of belladonna leaves. According to Kalow (*2*), the discovery of atropinesterase represents the first fully documented observation of a heritable modification of a pharmacological response. It is also known that the serum

of some but not all rabbits is capable of hydrolyzing cocaine (*1, 3*), but the enzyme involved has been much less extensively studied than atropinesterase. In this report we describe a polymorphic system of esterase isozymes in rabbit serum and show that the activity of atropinesterase and cocainesterase is localized within that system.

Horizontal electrophoresis in starch gels followed by histochemical staining of the gel slices for esterases was employed, after the original method of Hunter and Markert (*4*). Optimum resolution of the esterases was attained by using a gel buffer (*p*H 6.8) prepared by combining stock solutions A (10.5 g of monohydrated citric acid per liter of H_2O), B [23 g of tris (Sigma 7-9) per liter of H_2O], and C (1.2 g of lithium hydroxide and 11.8 g of boric acid per liter of H_2O) with H_2O, in a ratio of $3A:2B:4C:16H_2O$. The amount of starch (Connaught) varied by lot, but averaged 15 percent. Stock solution C (*p*H 8.1) was used in the electrode vessels.

Serum samples adsorbed onto paper strips (Whatman 3MM) were inserted into a cut made 5 cm from the cathodic end of the gels, and 150 volts were applied for 2 minutes, after which time the strips were removed and ice packs were placed on the surfaces of the gels. Electrophoresis was continued, by applying 350 volts, until the borate boundary had migrated 10 to 11 cm beyond the point of insertion. The dimensions of the gel frames and other details, including staining, are described elsewhere (see *5*).

Most of the staining was concentrated in a region of the gels just anodal to the albumins where it was possible to distinguish six phenotypes, as shown in Fig. 1. Except for occasional difficulties in distinguishing between phenotypes P and S, all rabbits were readily classifiable into one or another of the six phenotypes.

Data on the inheritance of the six phenotypes and their distribution in various populations of rabbits are in preparation (*6*). It is enough to mention here that the six phenotypes appear to be controlled by a series of five alleles, *A, F, M, P,* and *S* such that genotypes *AA, AM, AP,* and *AS* give rise to phenotype A, genotype *AF* gives rise to phenotype AF, genotypes *FF, FM, FP,* and *FS* give rise to phenotype F, genotype *MM* gives rise to phenotype M, genotypes *PP, PM, PS,* and *MS* give rise to phenotype P, and genotype *SS* gives rise to phenotype S.

In this report we focus attention primarily on isozymes A and S, the former restricted to phenotypes A and AF and the latter common to all phenotypes except M, as may be seen in Fig. 1. Zone A was the most rapidly and intensely staining of all, followed by zone S, when we used α-naphthyl acetate, β-naphthyl acetate, and α-naphthyl propionate as substrates. With α-naphthyl butyrate, zones A and S stained at about the same rate and to similar degrees of intensity. With the higher carbon substrates (α-naphthyl valerate through α-naphthyl caprate) the rate of staining of all zones was progressively slower, but zone S was always the most intensely staining.

Rather pronounced differential staining effects were observed when naphthol AS-D and AS-LC acetates were used as substrates. With naphthol AS-D, zone S stained well, and zones A and F, along with the intermediate zone of phenotype F (a zone also common to phenotype AF), stained moderately, whereas none of the zones of phenotype M, the minor zones of phenotypes P and S, and the leading zone of phenotype A were active. Also, an additional zone which migrated just behind zone S became evident in phenotypes AF and F. With AS-LC acetate, the only zones that stained were F and S and the intermediate zone of phenotype F. With both of these substrates the zone of "albumin" esterase, common to all phenotypes, became clearly visible. (The position of this zone is indicated in Fig. 1.)

The most pronounced effects were obtained when runs were performed on samples of serum that had been heated 1 hour at 56°C. This treatment inactivated zones A, F, the intermediate zone of phenotype F, and the leading zone of phenotype A, but had no effect on the activity of the other zones. In effect, the heat treatment converted phenotypes A, AF, and F to phenotypes that were indistinguishable from phenotypes P and S.

Atropinesterase was tested for in 268 samples of serum (99 type A, 17 type AF, 34 type F, 47 type M, 51 type P, and 20 type S) by means of a rapid qualitative colorimetric procedure adapted after a method described by Margolis and Feigelson (*7*). To 100 ml of a 1-percent solution of Noble agar (Difco) at 55°C the following were added: 1 g of atropine sulfate, 2 ml of a 0.16-percent aqueous solution of cresol red, and 0.1*N* NaOH, dropwise, until a purple color was attained. Two-milliliter aliquots of this solution were pipetted onto microscope slides. When the agar solidified, the slides were placed in individual petri dishes and kept in a refrigerator overnight. Seven wells (3 mm in diameter) were cut in each slide, and the plugs were removed by suction. Each well (capacity about 10 μl) was filled with a sample of test serum and the slides were kept at room temperature, readings being made at intervals of 3 and 6 hours. Hydrolytic activity was clearly indicated around the periphery of those wells where there was a color change from purple to yellow. There were 116 positive reactions, and these were limited to the 116 samples of phenotypes A and AF.

Mydriatic tests, adapted after the methods of Werner (*3*), were performed on 35 rabbits (11 type A, 3 type AF, 6 type F, 7 type M, and 8 type P). These tests were performed by adding 0.05 ml of a 2-percent solution of atropine sulfate in 0.9-percent saline to the eyes of each rabbit and measuring the time of recovery of the pupil reflex. There were 14 rabbits that showed the rapid recovery rate, and these were the 14 rabbits of phenotypes A and AF.

Thus it would appear that the atropinesterase activity of rabbit serum is limited to phenotypes A and AF where it is localized in (or is dependent on) one or, at the most, two isozymes, namely, zone A and possibly also the leading zone of phenotype A (Fig. 1).

The serums of 242 rabbits (81 type A, 14 type AF, 34 type F, 45 type M, 46 type P, and 22 type S) were screened for cocainesterase by means of a qualitative colorimetric procedure identical to the aforementioned method for atropinesterase except that 0.5 g of cocaine hydrochloride was used as substrate and 2 ml of a 0.16-percent aqueous solution of phenol red was used as indicator. We added NaOH to the mixture of agar, substrate, and indicator until a red color was attained. Readings of the tests were made at 4 and 8 hours. Because of the nonenzymatic hydrolysis of cocaine there was a progressive color change from red to yellow in the background agar which proceeded slowly during the 8-hour interval. However, at the periphery of the wells loaded with cocainesterase-negative serum the buffering capacity

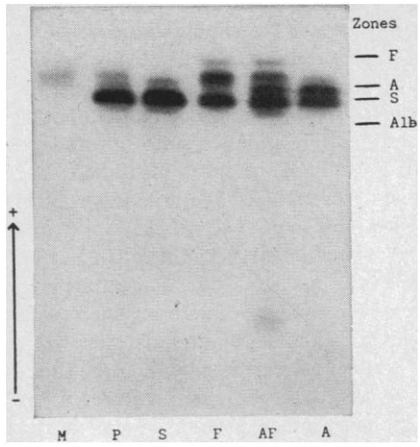

Fig. 1. Photograph of a gel slice stained in α-naphthyl acetate substrate solution and fixed in 50-percent methanol. This zymogram shows a system of rabbit serum esterases composed of six phenotypes labeled A, AF, F, S, P, and M. Atropinesterase acivity is limited to phenotypes A and AF and is believed to be localized in zone *A*. Cocainesterase activity is common to all phenotypes except M and is localized in zone *S*. *Alb* refers to the position of the albumin esterases which were clearly evident only when α-naphthol AS D and AS-LC acetates were used as substrates.

of the serum inhibited this color change throughout the 8-hour period. With cocainesterase-positive serum there were all degrees of color change ranging from those which progressed rapidly from red to light yellow to those which proceeded slowly from red to a reddish orange.

Among the 242 samples, there were 45 that failed to hydrolyze cocaine, and these nonreactors were restricted solely to the 45 rabbits of phenotype M. The only isozyme common to phenotypes A, AF, P, and S, and absent in phenotype M, is zone S (Fig. 1). Thus, it would appear that all the cocainesterase activity of rabbit serum is localized in isozyme S. Significantly, all 14 samples of phenotype AF and all 22 samples of phenotype S were classified as strong reactors, whereas weak reactions were observed only in phenotypes A, F, and P. These observations are consistent with the aforementioned genetic theory based on five alleles, because, according to that theory, all rabbits of phenotypes AF and S would be expected to possess a double dose of isozyme S, whereas some of the rabbits of phenotypes A, F, and P would be expected to be heterozygous for isozyme S and, therefore, would possess only a single dose of that isozyme.

With respect to both enzymes, three classes of rabbits were observed: those whose serums were positive for both enzymes (types A and AF), those whose serums were positive for cocainesterase but negative for atropinesterase (types F, P, and S), and those whose serums lacked both enzymes (type M). Ammon and Savelsberg (*1*) tested 22 samples of serum for both enzymes by means of a manometric method and, contrary to our observations, observed that some of the rabbits possessed atropinesterase but no cocainesterase. Although it is possible that such rabbits exist, we believe it is more likely that they overcorrected for nonenzymatic hydrolysis, thereby leading to the misclassification of some phenotypes.

In view of the foregoing observations, it is predictable that substrates may eventually be found that are hydrolyzed only by isozymes peculiar to phenotypes AF and F, namely, zone F and the intermediate zone of phenotype F. It is also predictable that the present results will lead to further experiments concerned with the biochemical, genetic (including ontogenetic and pharmacogenetic), and immunological aspects of atropinesterase and cocainesterase, especially as those two enzymes relate to the isozymes reported here. For example, it has been reported (*7*) that atropinesterase-negative rabbits produce isoprecipitins specific for atropinesterase when injected with serum from an atropinesterase-positive rabbit, and that the serum of rabbits which lack this enzyme contains no protein that is immunologically related to atropinesterase. The present results, along with those on the genetics of this six-phenotype system (*6*), would suggest that the isozymes of this system are closely related structurally and therefore should share common antigenic sites. With respect to hydrolytic properties, it has been reported that the enzyme in rabbit serum which hydrolyzes atropine is the same enzyme that hydrolyzes monoacetylmorphine, but Ellis (*8*) concluded that the activities of the plasma and liver of atropinesterase-positive rabbits on monoacetylmorphine, benzoylcholine, and atropine are due to three separate enzymes. We suspect that considerable tissue specificity of the isozyme patterns reported here will be revealed in subsequent studies and that such tissue specificity is responsible, at least in part, for the conflicting data. But irrespective of genetic, biochemical, and other considerations, we believe that knowledge of the six phenotypes described here will be helpful in clarifying the different responses of individuals to a variety of different drugs.

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Influenza Virus Effects on Cell Membrane Proteins

Abstract. *During infection by influenza virus, viral proteins become firmly attached to (or part of) host cell plasma membrane, nuclear membrane, mitochondrial, and microsomal membranes. Purified virus particles contain less than 1 percent host cell protein. Virus envelope proteins completely replace host membrane proteins in those discrete spots on the plasma membrane from which progeny virions bud out.*

The cellular membranes of mammalian cells are composed of an elaborate array of proteins of widely different molecular weights (*1*), and the lipoprotein membranous envelope of arboviruses is composed of a single protein associated with phospholipid (*2*). We determined the protein composition of influenza virus envelope and of the membranes of cells infected by influenza virus because this virus matures by budding from the host cell plasma membrane (*3*). Laver (*4*) has shown that there are three major protein components in purified influenza virions, and he has partially characterized the major structural components of the virus membrane—the hemagglutinin and the neuraminidase. Using