ATROPINESTERASE, A GENETICALLY DETERMINED ENZYME IN THE RABBIT

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Introduction.—Hereditary differences in enzyme activity are well known among both plants and animals, particularly in so far as they have to do with irregularities in pigment formation.[10, 13] In animals such differences are not restricted to pigment formation but have to do with many other physiological activities such as the xanthophyllase activity which determines the presence or absence of yellow color of body fat in the rabbit,[1, 11] the amylase activity in the digestive juices of silkworm larvae[7] and the uricase activity which determines the amount of nitrogen excreted as uric acid in the dog.[5] In man alkaptonuria, steatorrhea, hematoporphyria, pentosuria and cystinuria are other biochemical differences which are suspected to have a similar background.

Recently interest has been revived in the observation, first reported by Fleischmann[3] and subsequently confirmed by others in various parts of the world, that the blood of certain rabbits can destroy atropine while that of others cannot. Studies of some properties of the enzyme in the serums of those rabbits capable of hydrolyzing atropine have been carried out by Glick,[4] and Glick and Glaubach[5] have investigated the distribution of the atropinesterase among certain tissues in these animals. The possibility that atropinesterase in rabbit serum is an inherited factor was recognized by Levy and Michel[7] but no supporting data have been forthcoming. The present coöperative investigation is an attempt to determine the genetic properties of the enzyme.

Methods.—Blood samples of sufficient volume to provide 0.5 cc. of serum have been obtained from animals at three months of age or older of the proper matings in the laboratory at Brown, centrifuged to remove the cells and placed in the mail for examination within three days at Newark. The enzyme activity was determined by the manometric method employing the Warburg apparatus in the manner previously described.[4, 5] The activity was expressed as atropinesterase units per 100 mg. of serum. The unit is defined as the amount of enzyme required to liberate 1 c. mm. CO_2 in 300 minutes at 30° in a total volume of 4 ml. in the bicarbonate-Ringer medium containing a concentration of substrate (0.25% atropine sulphate) sufficient to achieve the maximum rate of hydrolysis.

Data and Discussion.—Preliminary examination of five unrelated families showed that about 55 per cent of the 181 rabbits examined possessed the

atropinesterase. This is a considerably higher proportion than previously obtained by other investigators and suggests the existence of racial differences, a fact that becomes more apparent when this population is separated into its constituent parts. Two of the families—III, a New Zealand White race which has been closely bred for more than 10 generations, and the *A* race of Castle, a small-sized multiple-recessive strain—appear to lack the enzyme entirely. The other three families possess it in a high proportion of the individuals. Family V, a pure chinchilla race, also closely bred for 12 generations, and family X, a more heterogeneous race originated by hybridization of the small race with other genetic stocks, produce both enzyme-possessing and non-enzyme-possessing offspring. In the first family, among 50 individuals tested the proportion was equal; in the second, 75 per cent of 87 animals examined were atropinesterase-producing. Only 14 individuals have been examined in family II*c*, of which three did not possess the enzyme. Of these families, III, V and X at least, show significant genetic differences. No entire family was found which does not produce some individuals lacking the enzyme.

The evidence as to the hereditary nature of these differences is as follows. In a total of 69 offspring obtained from parents lacking the enzyme, none have shown any trace of the enzyme. Parents possessing the enzyme, on the other hand, may or may not transmit the character to their offspring. If those which do possess it are paired with mates which do not, one of two alternatives follows. Either the offspring *all* possess the enzyme or they are about equally divided between those which possess it and those which do not. This indicates homozygosity in the first case and heterozygosity in the second case, of the parent possessing the character. Thirty-seven individuals have been obtained from the first type of mating, all of which were positive. In a population of 173 individuals obtained from the second type of mating, 88 possessed the enzyme in their blood and 85 did not, which is a close approximation to the equality expected from a monohybrid backcross. From known hybrids mated with each other, 68 young possessed the enzyme and 30 lacked it. This departure from the expected 3:1 ratio is not significant since it is less than twice the probable error.

From these results it appears that the ability to produce the enzyme is dominant over its absence. Dominance is probably not complete, however, since animals known to be heterozygous show a lower mean value of enzyme production than those which are homozygous. For 25 animals known to be heterozygous the mean value was 107 with a range of 52–174. For 4 animals known to be homozygous the mean value was 271 with a range of 232–348.

It is also interesting to note that the enzyme is not present at birth but first manifests itself at one to two months of age. Forty-five animals have been bled from the heart at birth and at monthly intervals two or three

times thereafter. Thirty-nine of these possessed at least one homozygous parent and hence could be expected to have the enzyme in their blood at three months of age. Six were from a heterozygous male and a non-enzyme mother. None of those examined at birth had demonstrable activity in their blood. Twelve out of 16 of the individuals of the first group, examined at the end of the first month, possessed the enzyme in substantial amounts and two others had a trace. All of them were active at subsequent examinations. Of the second group none possessed the enzyme at the end of the first month but one of them did show it at later examinations.

Similar quantitative differences and also differences in the age of production of the enzyme are apparent in the amylase of the digestive juice of the silkworm,[7] and in several enzymes of the pig.[8]

Through information supplied by Dr. C. I. Wright of the National Institute of Health, Bethesda, Md., who had previously discovered the enzy-

TABLE 1

EVIDENCE FOR GENETIC LINKAGE BETWEEN GENES *As* AND *E*

Backcross progeny from F^1 double heterozygote × double recessive

	NON-CROSSOVERS		CROSSOVERS		POPULATION
	E As	*e as*	*E as*	*e As*	
Coupling	10	12	4	4	30
	E as	*e As*	*E As*	*e as*	
Repulsion	4	5	1	2	12
Totals	31		11		42

The deviation from equality, 10, is 4.5 times the P.E., 2.19, and so clearly significant. The indicated crossover percentage is 26.2 ± 5.2.

matic hydrolysis of certain morphine derivatives,[12] there is reason to believe that the enzyme which hydrolyzes atropine may be identical with that which hydrolyzes monoacetylmorphine. Nineteen serums from our laboratory and 8 from his own have been examined by Dr. Wright. Each sample was examined for the hydrolysis of both substrates under the same conditions. All of the 17 individual bloods which hydrolyzed the one compound also hydrolyzed the other, whereas those of 10 individuals hydrolyzed neither. According to Dr. Wright, the order of activity is the same when the serums are arranged as to enzyme concentration for either "monoacetylmorphinease" or "atropinesterase." The initial rate of hydrolysis of the former is somewhat greater than for the latter but the time required for complete hydrolysis is approximately the same, due to the difference in the order of the two reactions.

For the gene responsible for atropinesterase production we shall use the symbol *As* and for its recessive allele, *as*. This gene apparently is borne

on the same chromosome of the rabbit as is the gene E for the extension of black pigment in the coat, for in matings between a double recessive individual and an F_1 double heterozygote, crossover recombinations are significantly fewer than non-crossovers, the two classes being 11 and 31, respectively (table 1), whereas equality would be expected if no linkage existed. Tentatively the genes As and E are regarded as members of a sixth linkage group of the rabbit. Segregation of both of these pairs of genes in this population is entirely regular when they are considered separately.

We regret that due to the national emergency, which has made it impossible for one of us to continue the work, a more accurate determination of the actual strength of this linkage association cannot be obtained at this time. In comparison, however, similar data have been obtained from crosses involving combinations of As and the agouti gene A and the color gene C. Nine and 8 offspring, respectively, have been obtained from these matings and the offspring are as equally divided between crossover and non-crossover combinations as possible.

Considering the population as a whole there is an obvious tendency for females to manifest greater enzyme activity than males, although no significant difference in the distribution of the character to the two sexes is manifest. Close scrutiny of the individual matings, however, reveals that in the backcross matings ($Asas \times asas$) this tendency is quite pronounced, the average male and female titers being 98 and 129, respectively, and there is a statistically significant tendency for females to possess the character more often than males. A similar situation occurs in the F_2 although less pronounced. The full meaning of this observation is not apparent. It seems probable, however, that possession of atropinesterase is not in itself a sex-linked character since in the same backcross population sufficient numbers have been obtained to indicate that it makes no difference whether the As gene is derived from the mother or the father. It seems more probable that this peculiarity is the result of a difference in the genetic milieu of the two sexes, perhaps acting secondarily through the medium of the sex hormones. It is interesting to compare these results with the similar behavior of cholinesterase in rats and mice in which age and also sex are important factors.[2]

Conclusion.—Rabbits which have in their blood serum an enzyme capable of hydrolyzing atropine (and monoacetylmorphine) inherit that peculiarity in a gene (As) borne in the same chromosome as the gene (E) for the extension of black pigment in the coat. The gene (As) is incompletely dominant, homozygotes producing the enzyme more effectively than heterozygotes. The enzyme is not present at birth but appears first at about one month of age, and tends to occur in greater concentration in females and to be demonstrable in a higher percentage of them than in males.

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NOTE ON THE TIME-INTENSITY FACTOR IN RADIOBIOLOGY

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In many radiobiological reactions the effect of a given dosage of radiation is found to depend on the "time-intensity factor," that is, to be a direct function of the intensity ("intensity effect") and to be lower when the treatment is intermittent than when it is continuous, the intensity remaining the same ("fractionation effect"). This phenomenon has been generally attributed to recovery of the biological material from the action of the radiation. The intensity effects have been determined by comparing the results of continuous irradiation with constant dose and different intensities. They have been the object of most experimental and theoretical investigations in this field, although they do not seem to supply, *per se*, crucial information on the course of recovery. In a recent study of the intensity effect on x-ray induced chromosomal aberrations in Tradescantia microspores,[1] one of us (L. D. M.) came to the conclusion, to be amplified and generalized in this note, that fractionation experiments, consisting of two high-intensity irradiations separated by a variable intermission, should be better suited to the investigation of the time-intensity factor. This is because in fractionation experiments radiation can usually be delivered in a short time, during which recovery is negligible, and recovery