INHERITED VARIATION IN THE DEHYDROGENASES OF DOVES (STREPTOPELIA). I. STUDIES ON 6-PHOSPHOGLUCONATE DEHYDROGENASE¹

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FILDES and PARR (1963) described inherited electrophoretic variation in human erythrocytic 6-phosphogluconate dehydrogenase (6-phospho-D-gluconate: NADP oxidoreductase: E.C. 1.1.1.44: 6-PGD, see International Union of Biochemistry 1964). Several papers have followed giving further genetic data (FILDES and PARR 1964; BOWMAN *et al.* 1966; GORDON, KERAAN and VOOLJS 1966; PARR 1966; DAVIDSON 1967) and comparing the enzymatic properties of some of the variants (CARSON *et al.* 1966). Inherited variation in enzyme activity is also described for this enzyme in man (PARR and FITCH 1964, 1967; BREWER and DERN 1964; PARR 1966; DERN *et al.* 1966). PARR (PARR 1966; PARR and FITCH 1967) has proposed that both classes of variation found in his investigations are controlled by one autosomal locus (symbol *PGD*).

Reports have appeared describing electrophoretic variation in 6-PGD in *Drosophila* (KAZAZIAN, YOUNG and CHILDS 1965; YOUNG 1966), the red blood cells of the deer mouse (SHAW 1965), the rat (PARR 1966) the pigeon (*Columba livia*) (YOUNG, cited by SHAW 1965 and personal communication 1967) and the cat (THULIN *et al.* 1967). It is autosomally inherited in all these organisms except *Drosophila*, where it is sex-linked. In the red cells of vertebrates, in squashes of whole *Drosophila*, and in the white cells of man, the electrophoretic pattern of the heterozygote exhibits three bands, the fastest and slowest being the same as the single band of the corresponding homozygotes. The intermediate band results from the union of the different polypeptides produced by the two unlike alleles, i.e. presumably the enzyme is a dimer. This type of variation has been found for a number of other enzymes (see SHAW 1965).

In this report, data are given on inherited variation in erythrocytic 6-PGD in a dove, *Streptopelia risoria*. The phenotype of two other *Streptopelia* species, *humilis* and *senegalensis*, and the phenotypes in F₁ *humilis/risoria* and *senegalensis/risoria* hybrids and the progeny of the first backcrosses of these hybrids to *risoria* are also described. YOUNG's finding (cited by SHAW 1965) of this polymorphism in *Columba livia* is confirmed and data are given on the electrophoretic phenotype of the enzyme in the tissues of *risoria* and *livia*.

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MATERIALS AND METHODS

Birds: The *livia* birds were from a mixed group of breeds obtained locally. The *risoria* were from a colony maintained in this laboratory. In addition to the *risoria* another group of birds, called here "*backcross risoria*," were used. These birds have the *risoria* genome with at least one red cell antigenic marker introduced from another species of *Streptopelia*. The genome of a backcross *risoria* bird consists of *risoria* genes except for the one or more red cell antigenic markers and those closely linked to them. For further information on these birds, see IRWIN (1966a, b) and STIMPELING and IRWIN (1960). The *humilis* and *senegalensis* birds were obtained from a dealer in California. The F_1 *humilis/risoria* hybrids, the F_1 *senegalensis/risoria* hybrids and the offspring of the backcross of both these hybrids to *risoria* were bred in this laboratory.

Gel electrophoresis: About 1–2 ml of blood was collected by brachial venipuncture into citrated saline (5 g NaCl, 17.4 g Na citrate, made up to 1 l with distilled water). Cells were washed three times with physiological saline (0.91% NaCl) and then one part cells were lysed with two parts of a solution of 4 mg NADP/100 ml of distilled water. The hemolysates were carefully centrifuged for 30 min at $25,000 \times g$ to remove gel-like material which formed immediately after lysis and caused an unreadable streaked enzyme pattern. Hemolysates were stored at -30°C until used. Freezing and thawing up to 6–8 times had no apparent effect upon enzyme activity or enzyme pattern.

The detection of the enzyme polymorphism by starch gel electrophoresis is a modification of the technique used by BOWMAN *et al.* (1966) for the polymorphism of the same enzyme in man. "Electro-Starch" (Otto Hiller, Madison, Wisconsin) was used. The gel was made of 72 g of starch, 24 ml of the stock buffer solution containing 0.09M Tris (hydroxymethylaminomethane) 0.5M boric acid and 0.02M EDTA (versene), pH 8.5, made up to 600 ml with distilled water. Before degassing the gel, 1.5 ml of a solution containing 4 mg NADP/ml was added to the molten starch. Each of the two cathode and two anode bridge trays contained 200 ml of a 0.0998M Tris-0.055M boric acid-0.0022M versene solution (SMITHIES 1965). To the forward cathode compartment, 2.5 ml (10 mg) of NADP solution was added. Without sufficient NADP in the gel and cathode vessel, blurred and weak enzyme patterns were obtained. Electrophoresis was carried out at 4°C for 18–20 hrs. The voltage drop was 5 v/cm. After slicing, the cut surface of the gel was stained with 100 ml of a solution containing 80 ml of 0.1M Tris-maleate buffer, pH 6.8, 20 ml 0.1M MgCl_2 , 4 mg NADP (Sigma), 25 mg 6-phosphogluconate trisodium salt (Pierce Biochemicals, Rockford, Ill. and Sigma Chemical Co., St. Louis, Mo.), 10 mg of phenazine methosulphate (Sigma), and 25 mg tetranitro blue tetrazolium (TNBT) or nitro blue tetrazolium (NBT). The gel and this solution were incubated at 37°C in the dark. The TNBT gave complete development of the enzyme after 1–2 hr as opposed to 4–5 hr with NBT (YOUNG 1966). TNBT detected weak bands more easily than NBT, but the photography was less satisfactory than for gels developed with NBT because TNBT gave more background. With TNBT, a navy blue color resulted; with NBT a purple color was found. Photography of the gels was carried out using Polaroid type 55 P/N film at $\frac{1}{4}$ sec and an aperture of $\frac{1}{9}$ with a green filter. Tissues were homogenized in distilled water or gel buffer with an Elvehjem-Potter piston (tissue:water being 1 g:2 ml). The resulting homogenate was spun at $25,000 \times g$ and the supernatant used for gel electrophoresis.

RESULTS AND DISCUSSION

Ten phenotypes were found in the erythrocytes of *risoria* and backcross *risoria*. They are described here as R1–1, R1–2, R1–3, R1–4, R2–2, R2–3, R2–4, R3–3, R3–4, and R4–4. Eight of these are shown in Figure 1. Mating data (Table 1) agree with the hypothesis (implied by the designation of the phenotypes) that the variation is controlled by one locus with four alleles, which we call PGD^{R1} , PGD^{R2} , PGD^{R3} , and PGD^{R4} .

[The method of presentation of the family data in Table 1 needs explanation.

FIGURE 1.—Patterns of eight of the ten phenotypes of 6-PGD of *risoria*. Symbols: 1 = R1-3, 2 = R1-1, 3 = R2-2, 4 = R1-4, 5 = R3-3, 6 = R3-4, 7 = R4-4, and 8 = R2-4. The enzyme presumably is a dimer. The starch gel electrophoresis is described in the text.

Its aim is to save space by reducing the number of columns needed to represent the phenotypes of the progeny when a locus with three or more codominant alleles is being investigated. First consider a locus with n alleles ($n > 3$). If a mating involves only one allele, i.e. it is between two like homozygotes, let that allele be i ; if it involves two, let them be i and j ; if three, i , j , and k ; if four, i , j , k , and l . Then every mating can be assigned to one of seven general classes (Table 2). This system of classification can be regarded as being based upon whether the matings are between two homozygotes, two heterozygotes, or a homozygote and a heterozygote, and the number of alleles involved. It is readily apparent that if these criteria are used, there are seven possible classes of matings. The maximum number of phenotypes (i.e. genotypes) which can result from any one mating is four, from classes 6 and 7. The total number of classes of progeny genotype under this system of classification is also seven, viz., ii , jj , ij , jk , jl , kl , and ik (Table 1). Three pairs of these, ii jl , jj jk , and ij il , cannot result from the same mating. Hence, these three pairs can share columns thus reducing to four the number of

TABLE 1

Mating data for the variant forms of the enzyme 6 phosphogluconate dehydrogenase in risoria
(see text for explanation of method of representing the data)

Class of mating	Number of matings	Decoding of matings, i.e. genotype of parents*				Genotype of progeny				Total progeny
		<i>i</i>	<i>j</i>	<i>k</i>	<i>l</i>	<i>ii</i> <i>jl</i>	<i>jj</i> <i>jk</i>	<i>ij</i> <i>il</i>	<i>ik</i> ..	
1. <i>ii</i> × <i>ii</i>	9	3	.	.	.	22	..	..	..	22
2. <i>ij</i> × <i>jj</i>	3	3	4	.	.	..	..	5	..	5
3. <i>ij</i> × <i>ii</i>	14	3	4	.	.	25	..	19	..	44
	3	3	1	.	.	3	..	5	..	8
	3	4	3	.	.	11	..	5	..	16
4. <i>ij</i> × <i>ij</i>	7	3	4	.	.	8	4	15	..	27
	1	2	3	.	.	0	0	1	..	1
5. <i>ii</i> × <i>jk</i>	1	1	3	4	..	..	..	2	4	6
	1	2	3	4	.	..	..	1	1	2
	2	3	1	4	.	..	..	1	2	3
	1	4	1	4	.	..	..	4	6	10
6. <i>ij</i> × <i>ik</i>	2	3	1	4	.	2	0	0	2	4
	1	3	2	4	.	1	0	1	3	5
	1	4	2	3	.	0	2	0	4	6
7. <i>ij</i> × <i>kl</i>	1	3	4	1	2	0	2	2	5	9

Note that the figures in italics under decoding of mating are abbreviated gene symbols.
* 1 = *PGD^{R1}*, 2 = *PGD^{R2}*, 3 = *PGD^{R3}* and 4 = *PGD^{R4}*.

columns necessary to represent the progeny. The particular mating involved (Table 1) can be indicated by writing the alleles in the appropriate columns under the heading "Decoding of mating". For example, in the first line in mating class 5, *PGD^{R1}* = *i*, *PGD^{R3}* = *j*, *PGD^{R4}* = *k*. The mating is R1-1 × R3-4 and from it came 2 R1-3 and 4 R1-4 progeny.]

The four *risoria* homozygotes each possessed one strong major band. The heterozygotes (R1-3, R1-4, R2-3, R2-4, and R3-4) possessed the major bands of their respective homozygotes and a "hybrid" band of intermediate mobility (Figure 1). This is to be expected if the enzyme is a dimer. The heterozygote R1-2 probably possesses three major bands but they would be so close together

TABLE 2

Classes of kinds of mating for a co-dominant multi-allelic locus

Symbolic representation of matings	Description of parental genotypes	Number of alleles
1. <i>ii</i> × <i>ii</i>	like homozygotes	1
2. <i>ii</i> × <i>jj</i>	unlike homozygotes	2
3. <i>ii</i> × <i>ij</i>	homozygote-heterozygote	2
4. <i>ij</i> × <i>ij</i>	like heterozygotes	2
5. <i>ii</i> × <i>jk</i>	homozygote-heterozygote	3
6. <i>ij</i> × <i>ik</i>	unlike heterozygotes	3
7. <i>ij</i> × <i>kl</i>	unlike heterozygotes	4

FIGURE 2.—Diagrammatic representation of the position and relative intensity of the bands of the four homozygotes of *risoria* (R), of two homozygous types of *livia* (L1-1 and L2-2), and the indistinguishable type in *humilis* (H1-1) and *senegalensis* (S1-1).

that they could not be separated. However, this phenotype can be distinguished as a band three times as thick as any major band.

The major bands were associated with other less intensely staining minor bands. The position and relative intensity of those found in the homozygotes are shown in the diagram of Figure 2. R1-1 had a slower minor band, none were detected in R2-2; R3-3 had a faster one, whereas R4-4 had two faster ones. (The L, H and S phenotypes of *livia*, *humilis* and *senegalensis* will be discussed below.)

The hybrid major bands of R1-4 and R2-4 also had slightly faster minor bands associated with them, not visible in the figures. The other hybrid major bands may also have possessed them, but these were not detected. The relative mobilities of the major bands of R1-1, R2-2, R3-3, R4-4, and of the two minor bands of R4-4 were the same in starch gels with concentrations of 10, 12, and 14 g starch per 100 ml buffer. Thus, there appear to be no large size differences between any of the proteins which produce these bands (SMITHIES 1964).

Population data are given (Table 3) for the *risoria* and backcross *risoria*. In both, there is agreement with Hardy-Weinberg expectations. The gene frequencies of the two groups are similar (Table 4), although the difference between those of PGD^{R1} is significant (χ^2_1 , with Yates' correction, = 7.68).

The patterns for *senegalensis* (S1-1) and *humilis* (H1-1) were indistinguishable from each other, being composed of a major band slightly slower than the major band of R4-4 and a slightly faster minor band (Figure 3). The combination of PGD^{S1} or PGD^{H1} with PGD^{R1} , PGD^{R2} , and PGD^{R3} , resulted in a pattern similar to the R1-4, R2-4 and R3-4 patterns with the slowest major band being slightly slower than the major band of R4-4, i.e. of the same mobility as H1-1 and S1-1. These patterns are called R1-S1, R2-S1, etc. The combination of PGD^{R4} with

TABLE 3

Population data for 6-phosphogluconate dehydrogenase variants in risoria

	<i>risoria</i>		<i>risoria</i> backcrosses†	
	Observed	Expected	Observed	Expected
R1-1	2	0.19	0	0.88
R1-2	0	0.29	1	0.54
R1-3	3	5.90	9	6.91
R1-4	2	2.44	3	3.79
R2-2	1	0.11	0	0.08
R2-3	4	4.59	1	2.13
R2-4	1	1.89	2	1.17
R3-3	48	46.90	11	13.55
R3-4	40	38.70	19	14.88
R4-4	8	7.98	2	4.00
Total	109	109.00	48	48.00
χ^2 *	1.81		3.95	

* For agreement with Hardy-Weinberg expectations; obtained by pooling the classes R1-1, R1-2, R2-2, R1-4, and R2-4.

† See MATERIALS AND METHODS for a description of these.

PGD^{S1} or PGD^{H1} (R4-S1), although similar to S1-1 and H1-1, could be distinguished from them.

Table 5 gives the numbers of the various types found in the F_1 and progeny of backcrosses of *senegalensis/risoria* and *humilis/risoria* hybrids to *risoria*. The parents of individual birds of the F_1 and backcross generations were not available. Accordingly the data for these groups of birds are presented as population data. The data in Table 5 and the electrophoretic patterns obtained suggest, as implied also by the designation, that the various genes in *risoria* are allelic to the PGD^{S1} and PGD^{H1} genes in these species.

Our results for *C. livia* confirm those of YOUNG (cited by SHAW 1965). Among 58 pigeons, 50 were found which had the same single major band, with one faster minor band (L1-1, see Figure 2). The remaining eight had a three-banded pattern (L1-2, not shown in Figure 2) with the fastest band corresponding to the major band of L1-1. In a sibship whose parents were not examined, a third type (L2-2, see Figure 2) with a major band corresponding to that of the slowest band in the three-banded type was found. This third type also had a faster minor band.

TABLE 4

Gene frequencies for 6-phosphogluconate genes in risoria

	<i>risoria</i>	<i>risoria</i> backcrosses
PGD^{R1}	0.041	0.135
PGD^{R2}	0.032	0.042
PGD^{R3}	0.656	0.531
PGD^{R4}	0.271	0.292

TABLE 5
6-PGD phenotypes of progeny from interspecific and hybrid matings

Matings	Progeny													Ratio of <i>risoria</i> to hybrid phenotypes
	<i>risoria</i> phenotypes							Hybrid phenotypes, (<i>senegalensis</i> × <i>risoria</i>) (<i>humilis</i> × <i>risoria</i>)						
								or			or			
	R1-1	R1-3	R1-4	R2-3	R3-3	R3-4	R4-4	S1-R1 or H1-R1	S1-R2 or H1-R2	S1-R3 or H1-R3	S1-R4 or H1-R4			
<i>senegalensis</i> × <i>risoria</i>	..	..	..	..	..	..	..	..	..	3	3		0:6	
(<i>F</i> ₁ - <i>senegalensis</i> × <i>risoria</i>) × <i>risoria</i>	0	2	1	2	2	9	1	1	..	17	10		17:28	
<i>humilis</i> × <i>risoria</i>	..	..	..	..	..	..	..	..	..	3	..		0:3	
(<i>F</i> ₁ - <i>humilis</i> × <i>risoria</i>) × <i>risoria</i>	2	1	..	..	3	2	..	1	1	7	1		8:10

FIGURE 3.—Patterns of 6-PGD in various tissues of an R1-3 *risoria* bird. BM = bone marrow, RC = red cell, L = liver, K = kidney, B = brain, H = heart. The gel was removed from the staining solution before the red blood cell had stained in order to avoid overstaining the tissue samples. On the original gel faint bands of the red blood cell can be seen having the mobility of the first three bands in the bone marrow sample. Note that the kidney and liver have essentially the same pattern as the other tissues but with each band slightly faster. Bone marrow, liver and brain possess slower minor bands not seen in the red blood cell.

By analogy with the results in *risoria*, it is reasonable to suggest that the differences are controlled by two alleles, PGD^{L1} and PGD^{L2} . The major band of L1-1 was just behind that of R3-3 and that of L2-2 just behind R4-4. One generic hybrid (F_1 -*livia/risoria*) had a R2-L2 phenotype, with the intermediate band. A direct comparison of L2-2 with H1-1 or S1-1 was not made, but these phenotypes would probably be indistinguishable from one another.

Very limited data are available suggesting that two blood group loci (*ch-4*, *ch-8*) which are recognized in the backcross *risoria* (IRWIN 1966a, b) are not linked to the *PGD* locus.

The enzyme patterns in a variety of tissues of adults were examined for six

risoria of various phenotypes, two backcross *risoria*, and eight *livia*. The results, which are shown in Figure 3, are summarized as follows: With the exceptions to be described, the same basic pattern occurred in erythrocytes and in tissues. Thus, the *PGD* locus appears to be active in all the tissues studied. Strong *PGD* activity was found in bone marrow, liver, kidney, eye, brain, heart, testis, ovary, thymus, spleen, spinal cord, lungs, and thyroid. All these tissues had stronger activity than the erythrocytes. Weaker activity was found in the leg and breast muscles, and none at all in the plasma. (Not all tissues were examined in all birds.) Some tissues, marrow, lungs, brain, spleen, testis and ovaries showed a slower minor band for each major band. These slower bands were not seen in the erythrocytic enzyme pattern. In some samples of a few tissues (marrow and liver) a second slower minor band was seen. The liver and kidney of *risoria* and backcross *risoria* and the liver of *livia* had a *PGD* pattern essentially like that of other tissues, but with each band slightly faster (see Figure 3).

We have no information on the significance of the minor bands which are associated with the major bands. However, AJMAR *et al.* (1968) have shown that a metabolite of NADP, 2-phosphoadenine diphosphate ribose, may bind to and alter the electrophoretic mobility of human red cell 6-PGD, i.e. if this substance were present in birds at variable concentrations, it might cause the appearance of additional bands. In some tissues, particularly liver, but also brain and kidney, a "nothing dehydrogenase" was found. This was probably alcohol dehydrogenase (SHAW and KOEN 1967; BEUTLER 1967). It possessed about half the mobility of the major band of R4-4 (Figure 3). Omission of 6-phosphogluconate from the reaction mixture resulted in no 6-PGD activity, but gave the same degree of "nothing dehydrogenase" activity.

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SUMMARY

Inherited polymorphisms in the 6-phosphogluconate dehydrogenase (6-PGD) of doves (*Streptopelia risoria*) and pigeons (*Columba livia*) are described. There is one locus (*PGD*) in each species, with four alleles in *risoria* and two in *livia*. The *PGD* locus also controls the difference between the various *risoria* phenotypes and the single phenotype of two other *Streptopelia* species, *senegalensis* and *humilis*. The electrophoretic patterns suggest that the enzyme is a dimer. The *PGD* locus is active in all tissues, although there are some differences among the tissues in the phenotype of the enzyme. A new method is used for the compact presentation of family data for this multi-allelic codominant locus.

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