gauche est féminisé, au moins en partie; groupe 6: embryons femelles où les 2 canaux de Müller manquent en tout ou en partie.

Seuls les embryons des groupes 5 et 6 peuvent être considérés comme intersexués (Figure 2). La présence de tronçons mülleriens chez un hôte mâle, si elle n'est liée à la féminisation, au moins partielle, du testicule gauche, n'est pas un caractère intersexuel, l'absence du canal de Müller du seul côté opéré, chez un hôte femelle, non plus[1,3].

A l'intérieur de ces 6 groupes, selon l'espèce du transplant, selon qu'il a été retrouvé ou non ou selon son sexe, les hôtes se répartissaient comme l'indique le Tableau II.

On voit que les 37 hôtes intersexués étaient tous porteurs d'un transplant, sauf 4 – les intersexués mâles porteurs d'un transplant ovarien, excepté 3, les intersexués femelles porteurs d'un transplant testiculaire, excepté 1. En ce qui concerne ces 4 hôtes intersexués sans transplant, nous savons, grâce aux transplants correspondants retrouvés, que 2 d'entre eux, les 2 mâles, avaient reçu un transplant ovarien. Nous pouvons donc penser – et notre expérience antérieure[1,3,4], ainsi que celle de WOLFF[5], nous y encourage – qu'un transplant ovarien était présent chez le troisième hôte mâle intersexué et un transplant testiculaire chez l'hôte femelle intersexué, même si nous n'avons pas su les découvrir. Il nous faut encore signaler que les transplants retrouvés chez les hôtes intersexués n'appartenaient pas tous à la catégorie de ceux qui étaient parfaitement sains.

Conclusion et discussion. Nous établissons donc qu'un transplant testiculaire d'embryon de Perdrix ou de Pintade masculinise l'embryon de Poulet femelle et qu'un transplant ovarien d'embryon de Perdrix, Caille ou Pintade féminise l'embryon de Poulet mâle. Nous démontrons ainsi l'activité hormonale des gonades embryonnaires des 2 sexes de Perdrix et de Pintade et de l'ovaire embryonnaire de Caille. Les hormones sexuelles embryonnaires des 4 espèces de Phasianidés étudiées, Poule, Perdrix, Caille et Pintade, étant douées d'activité interspécifique, on peut penser qu'elles sont chimiquement identiques. Grâce à l'extrême sensibilité des méthodes radiochromatographiques[6-8], le problème pourra être abordé expérimentalement. Signalons, pour terminer, que l'activité hormonale de l'ovaire embryonnaire de Caille avait déjà été démontrée par HAFFEN[9], grâce à la culture in vitro.

Summary. When indifferent gonads from red-legged partridges, Japanese quail or pintado embryos were transplanted into the coelomic cavity of early chick embryos, they differentiated into ovaries and testes, regardless of the sex of the host. Testicular transplants brought about the retrogression of both Müllerian ducts in female hosts, and ovarian transplants the feminization of the left testis in male hosts. This demonstrates hormonal activity of the embryonic gonads in the 3 species under study. Since the embryonic sex hormones show interspecific activity, they may be of identical chemical nature.

H. AKRAM et J.-P. WENIGER[10]

Laboratoire de Zoologie et d'Embryologie expérimentale, 67 Strasbourg (France), 3 mars 1967.

[3] J.-P. WENIGER, Experientia *23*, 225 (1967).
[4] W. MALINOWSKA et J.-P. WENIGER, Experientia *21*, 91 (1965).
[5] ET. WOLFF, Archs Anat. microsc. Morph. exp. *36*, 69 (1946–1947).
[6] L. CEDARD et K. HAFFEN, C. r. hebd. Séanc. Acad. Sci., Paris *263*, Série D, 430 (1966).
[7] J.-P. WENIGER, J.-D. EHRHARDT et B. FRITIG, C. r. hebd. Séanc. Acad. Sci., Paris *264*, Série D, 838 (1967).
[8] H. AKRAM et J.-P. WENIGER, C. r. hebd. Séanc. Acad. Sci., Paris *264*, Série D, 1806 (1967).
[9] K. HAFFEN, C. r. hebd. Séanc. Acad. Sci., Paris *259*, 882 (1964).
[10] Avec la collaboration technique de A. ZEIS.

Mouse Hemoglobin: Chain Composition Multiple Electrophoretic Bands

The generally available inbred strains of mice are divisible into 2 groups, depending upon whether their hemoglobin exhibits a single or a multiple banded electrophoretic pattern[1,2]. Previous investigations have confirmed that the difference between these groups is determined by a single genetic locus, designated the Hb locus[3]. The 2 different genes at this locus are known as 'single' and 'diffuse'[1]. The hemolyzate prepared from mice homozygous for 'diffuse' has been variously reported as containing from 2 to 5 or 6 different hemoglobins[4-9]. The interest inherent in the situation where an apparently individual genetic locus is capable of controlling simultaneously several different hemoglobins prompted this investigation of the 'diffuse' hemoglobin pattern.

DBA/1J and C57BL/6J mice were used as the source of 'diffuse' and 'single' hemoglobin specimens respectively. Hemolyzates were prepared within 24 h of the time of blood letting and stored at 4 °C for periods up to 2 weeks. Immediately prior to electrophoresis (either in starch block or starch gel), the hemolyzate was converted to the cyanmethemoglobin form.

Electrophoresis of the whole hemolyzate from DBA/1J in starch gel (*Tris*-EDTA-Borate buffer, pH 8.9[10]) demonstrated the presence of 4 bands, of which the slowest was unusually broad, as if composed of several overlapping zones (Figure 1, pattern 4). These 4 bands will be referred to here as A, B, C and D, in order of decreasing anodal mobility. When dithiothreitol (DTT),

[1] H. M. RANNEY and S. GLUECKSOHN-WAELSCH, Ann. hum. Genet. *19*, 269 (1955).
[2] E. S. RUSSELL and P. S. GERALD, Science *128*, 1569 (1958).
[3] S. GLUECKSOHN-WAELSCH, H. M. RANNEY and B. F. SISKEN, J. clin. Invest. *36*, 753 (1957).
[4] J. R. MORTON, Nature *194*, 383 (1962).
[5] H. S. RHINESMITH, H. H. LI, B. L. MILLIKIN and L. C. STRONG, Analyt. Biochem. *8*, 407 (1964).
[6] J. ROSA, G. SCHAPIRA, J. C. DREYFUS, J. DE GROUCHY, G. MATHE and J. BERNARD, Nature *182*, 947 (1958).
[7] W. WELLING and D. W. VAN BEKKUM, Nature *182*, 946 (1958).
[8] J. J. HUTTON, J. BISHOP, R. SCHWEET and E. S. RUSSELL, Proc. natn. Acad. Sci. USA *48*, 1505 (1962).
[9] T. N. MEHROTRA and G. CARDINALI, Acta Genet. med. Gemell. *13*, 185 (1964).
[10] O. SMITHIES, Vox Sang. *10*, 359 (1965).

a potent reducing agent, was added to the hemolyzates, band D disappeared and band A decreased in relative intensity (Figure 1, pattern 5). This behavior of band D is consistent with that reported by others; band D presumably results from the polymerization (dimerization?) of hemoglobin molecules through the formation of disulfide bonds[11]. The persistence of 3 definite bands in the presence of reducing agent suggested that there might be 3 genetically different hemoglobins in these animals, despite the identification of only 2 components after resin column chromatography[8].

The three-banded pattern noted in starch gel (Figure 1, pattern 5) could be reproduced by starch block electrophoresis[12] using *Tris*-EDTA-Borate buffer[10] to which DTT was added (10 mg/l). The 3 bands were individually eluted and concentrated by pressure dialysis at 4 °C[13]. After repeat starch block electrophoresis of the isolated fractions, components of high purity were obtained, as judged by starch gel electrophoresis. No interconversion of components A, B, and C during the second electrophoretic run was detected, contrary to the findings of a previous report[14].

The purified components were next examined by urea-starch gel electrophoresis[15] using the *Tris*-EDTA-Borate buffer. The hemoglobins were converted to globin by precipitation with zinc acetate (final concentration of Zn^{++} was 20 m*M*/l) followed by extraction of the heme (and zinc) from the precipitate with acid-acetone. Components A, B, and C each showed the presence of 2 major bands, presumably corresponding to α and β chains. The α chain bands were of identical mobility in all 3 components.

The β chain of each component was isolated by chromatography of the globin on carboxymethylcellulose at pH 6.8, using a linear gradient of sodium phosphate (0.003–0.035 *M*) in 8 *M* urea containing DTT (100 mg/l)[16]. The urea was removed from the effluent fractions by passage through a column of Sephadex G-25 and the isolated chain was recovered by lyophilization. The isolated β chain of component A had the same mobility in urea-starch gel as that of B (Figure 2). The similarity of the β chains of components A and B was further tested by examination of the peptides obtained by cleaving the chains at the methionyl residues with cyanogen bromide[17]. The peptides were precipitated by the addition of acid-acetone. The precipitate was dissolved in 8 *M* urea containing β-mercaptoethanol (2%, v/v) and subjected to

[11] A. RIGGS, Science *147*, 621 (1965).
[12] P. S. GERALD and L. K. DIAMOND, Blood *13*, 61 (1958).
[13] F. PEETOOM and P. S. GERALD, Clinica chim. Acta *10*, 375 (1964).
[14] J. R. MORTON, Genet Res. *7*, 76 (1966).
[15] A. E. CHERNOFF, N. PETTIT and J. NORTHROP, Blood *25*, 646 (1965).
[16] J. B. CLEGG, M. A. NAUGHTON and D. J. WEATHERALL, Nature *207*, 945 (1965).
[17] E. GROSS and B. WITKOP, J. biol. Chem. *237*, 1856 (1962).

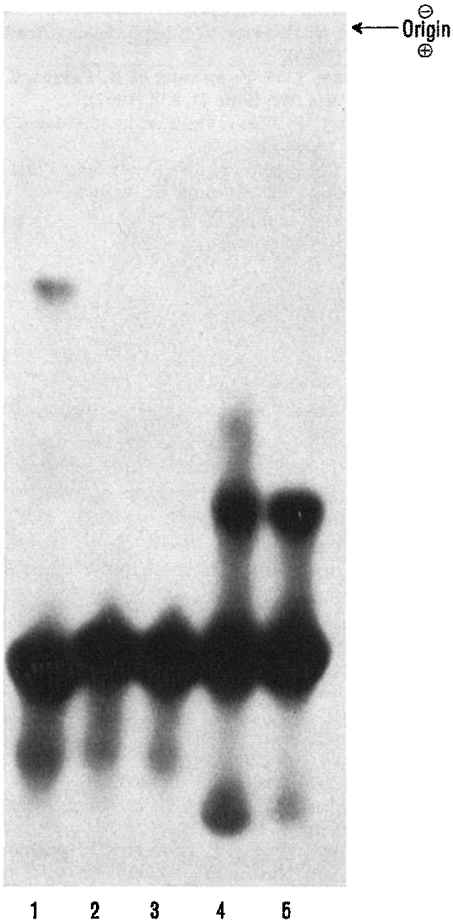

Fig. 1. Electrophoretic pattern in starch gel at pH 8.9 of whole hemolyzates (1) human, (2) C57BL/6J, (3) C57BL/6J, with added dithiothreitol, (4) DBA/1J and (5) DBA/1J, with added dithiothreitol.

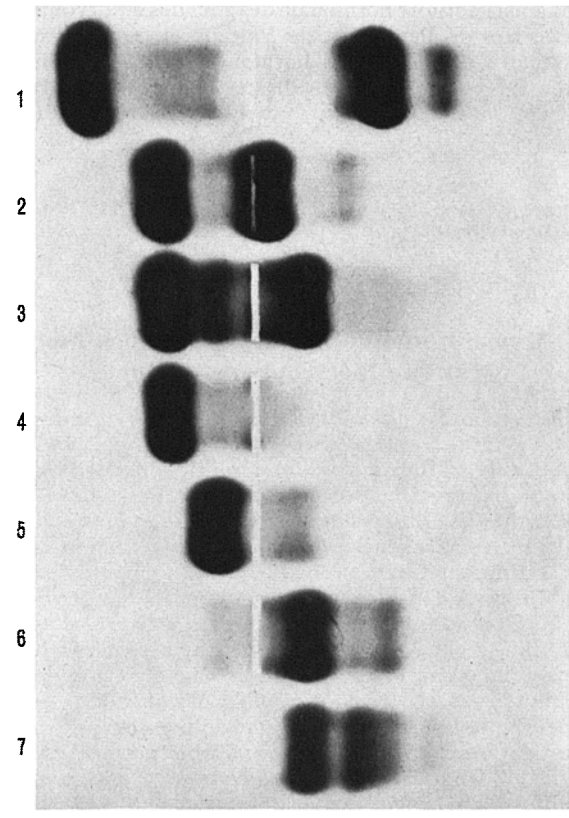

Fig. 2. Electrophoresis in urea-starch gel of globins. (1) Whole human, (2) whole C57BL/6J, (3) whole DBA/1J, (4) isolated α chain, DBA/1J, (5) isolated β chain from band C, DBA/1J, (6) isolated β chain from band B, DBA/1J and (7) isolated β chain from band A, DBA/1J.

electrophoresis in urea-starch gel. The electrophoretic patterns obtained with peptides from the 2 β chains were essentially identical, confirming the impression obtained from electrophoresis of the whole chains. The difference in mobility of the whole hemoglobins (components A and B) is thus not believed to represent a difference in amino acid sequence.

The isolated β chain of component C showed a major band with more cathodal mobility in urea-starch gel than that of component B (Figure 2), consistent with the mobility difference between the whole hemoglobins. The patterns obtained after cyanogen bromide cleavage of the β chains of components B and C were markedly different suggesting that there are multiple differences in their amino acid sequence. These findings are consistent with the results of HUTTON et al.[8] who isolated 2 components from hemolyzates of the 'diffuse' type (AKR and FL mice) by Amberlite CG-50 column chromatography. These workers identified at least 3 points of difference between the β chains of these 2 components by 'fingerprinting' the soluble tryptic peptides.

Despite the multiple banded nature of the hemoglobin electrophoretic pattern obtained with hemolyzates from mice homozygous for 'diffuse', there appear to be only 2 components with differing amino acid sequences. These components have identical α chains and vary only in their β chains[18].

Résumé. L'hémoglobine des souris de type DBA/1J apparaît à l'électrophorèse sous forme de 4 bandes. En les séparant par l'électrophorèse en gel d'urée-amidon, on ne trouve qu'une seule ligne de globine α et deux lignes de globine β. Les taches se montrant à l'électrophorèse après traitement au bromide cyanogène sur les chaînes peptides β suggèrent plusieurs différences entre les 2 séquences d'acides aminés.

E. SCHWARTZ and P. S. GERALD

Department of Pediatrics, Harvard Medical School and Children's Hospital Medical Center, Boston (Massachusetts 02115, USA), 9th June 1967.

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Correlation between Sensitivity to Trypaflavine and DNA Base Composition in Mutants of *Bacterium paracoli* 5099

A few rare mutants of *Bacterium paracoli* with the hereditary impairment of respiration were induced by urethane and by UV-radiation. The functional inefficiency of their respiratory system was accompanied by characteristic alterations in cytochromes of the mutant cultures[1]. One of these mutants was later studied in greater detail, and it was observed that guanine-cytosine content in DNA (% GC) of the mutant attained 70%, as compared with 55% of the parent culture[2]. Some other mutants, which form small colonies but do not reveal any defects in respiration, also do not reveal any alterations in GC content of DNA as compared with the parent strain. Another important observation is concerned with the fact that the mutant with altered GC content is about 100 times more sensitive to the action of trypaflavine, whereas mutants with small colonies and normal GC content do not differ significantly from the parent culture in their sensitivity to this aminoacridine[3]. If this correlation between drastic increase of sensitivity to trypaflavine and DNA base composition in mutants of *B. paracoli* has some real significance, it could then be exploited for the development of a new technique for isolation of mutants with altered GC content, which represent extremely rare events. This possibility was explored by us in experiments described below.

Mutants with small colonies were induced by UV-radiation at 2540 Å, with a radiation intensity of 11.53 erg/sec transmitted to each mm². *B. paracoli* 5099 was obtained from Type Culture Collection of U.S.S.R. (State Control Institute of Medical Biological Preparations of the Ministry of Health, Moscow). Bacteria grown on nutrient agar at 37 °C for 24 h were suspended in water to the density 10^9 cells/ml, poured into dishes in layers 1 mm thick, and irradiated for various intervals of time between 45 and 105 sec. Under given conditions the

[1] G. F. GAUSE, Nature *182*, 97 (1958).
[2] G. G. GAUSE, N. P. LOSHKAREVA, I. B. ZBARSKY and G. F. GAUSE, Nature *203*, 598 (1964).
[3] G. V. KOCHETKOVA, M. K. KUDINOVA, L. P. ZIMENKOVA and M. V. BIBIKOVA, Mikrobiologiya *33*, 587 (1964).

Table I. Growth of some small colony mutants of *B. paracoli* 5099 on gradient agar plates with trypaflavine

Strain	Irradiation time (sec)	Concentrations of trypaflavine in the upper layer of gradient agar plate, μg/ml										
		1000	500	250	125	62	31	16	8	4	2	1
Parent	None	–	+	+	+	+	+	+	+	+	+	+
Mutants												
168	75	–	–	–	–	–	–	–	–	–	+	+
975	45	–	–	–	–	–	–	–	–	+	+	+
1008	75	–	–	–	–	–	–	–	–	–	+	+
1041	90	–	–	–	–	–	–	–	–	–	–	+
1055	60	–	–	–	–	–	–	–	–	–	+	+