Genet. Res., Camb. (1962), **4**, *pp.* 240–247
With 1 *text figure and* 1 *plate*
Printed in Great Britain

Serum transferrins in Merino sheep

By G. C. ASHTON
Cattle Research Laboratory, C.S.I.R.O., Rockhampton, Queensland, Australia

AND

K. A. FERGUSON
Ian Clunies Ross Animal Research Laboratory, C.S.I.R.O., Prospect, New South Wales, Australia

(*Received* 14 *September* 1962)

1. INTRODUCTION

Polymorphism of the iron-binding β-globulins, also called transferrins and siderophilins, has been described in numerous mammalian species, including man (Smithies, 1957), cattle (Ashton, 1957; Hickman & Smithies, 1957), sheep (Ashton, 1958*a*), goats (Ashton & McDougall, 1958; Millson & Pattison, 1961), horses (Ashton, 1958*b*), pigs (Ashton, 1960*a*; Kristjansson, 1960), mice (Ashton & Braden, 1961; Cohen, 1961; Shreffler, 1961), chimpanzees (Boyer & Young, 1960; Buettner-Janusch, 1961), monkeys (Lai & Kirk, 1960; Blumberg, 1960; Goodman & Poulik, 1961), reindeer (Gahne & Rendel, 1961), and deer (Lowe & McDougall, 1961). In each species the genetic mechanism, where established, has proved to be essentially the same. The polymorphism is due to multiple autosomal allelomorphs exhibiting co-dominance, each allele causing the production of two or more protein staining zones in starch-gel after electrophoresis. The number and relative staining intensity of the zones produced by an allele are a characteristic of the species. In cattle and buffalo (Ashton, Jenkins & Tulloch, unpublished data) each allele produces four zones. Three zones are produced for each allele in pigs, mice, reindeer and horses. Two zones are produced in humans, chimpanzees, sheep and goats. No case is known where only one zone is formed. The reasons for this multiplicity of zones from one allele has been discussed by various authors (e.g. Ashton & Braden, 1961; Cohen, 1961; Patras & Stone, 1961).

English breeds of sheep examined previously (Ashton, 1958*a*) have shown five β-glubulin alleles. Staining with nitroso-R salt has shown that these are iron-binding proteins and hence transferrins. Examination of merino sheep has shown additional transferrins which are described in this paper.

2. MATERIALS AND METHODS

(i) *Starch gel electrophoresis*

For routine examination the serum proteins of sheep have been examined in an apparatus described elsewhere (Ashton, 1957, 1960*b*), which is a simple modification

of that described originally by Smithies, (1955). A discontinuous tris-citric buffer system (Poulik, 1957) as modified by Ferguson & Wallace (1961) was employed.

Some sera were also examined under high-voltage conditions in an apparatus which will be described elsewhere (Ferguson, unpublished data). The separation of transferrin types illustrated in Plate I was obtained with this apparatus.

Samples of serum for typing were run on the same gel as one or more reference samples of defined transferrin type.

(ii) *Serum samples*

Serum samples were obtained from four main sources:

(i) 225 samples from Tasmania from two of the original studs (Kenilworth and Valleyfield) on which the Tasmanian fine-woolled Merino was founded;

(ii) 1356 samples from Merinos, and 'Merinos' with some Border Leicester ancestry, at the National Field Station, 'Gilruth Plains', Cunnamulla, Queensland;

(iii) samples from the F. D. McMaster Field Station, Badgery's Creek, N.S.W., as follows: sera from thirty-six ewes purchased from the Pepping commercial flock used to establish the Badgery's Creek inbred families; samples from thirty-five animals from inbred sires and unrelated ewes of mixed Peppin origin; and samples from thirteen ewes from two of the inbred families;

(iv) 298 samples from a stud flock of Pepping Merinos in Western Central Queensland.

(iii) *Nomenclature of transferrin phenotypes*

The nomenclature of transferrin polymorphism is complicated by the multiple zones produced by each allele and by the ever-increasing number of newly recognized alleles in all species examined.

The fact that a transferrin allele gives rise to more than one zone in starch gel was first reported by Ashton & McDougall (1958) from a comparison of paper and starch gel electrophoretic results with cattle, sheep and goat sera. To facilitate description of newly discovered phenotypes in sheep the term 'zone pair' has been used to describe the two zones produced in starch-gel by a sheep transferrin allele. Any new allele can then be defined in terms of the mobility of the zone pair it produces in starch-gel under the stated conditions, relative to established reference sera. (In the same way species with alleles producing three zones might be considered to have transferrin zone trios, and species with four zones, transferrin zone quartets.)

The naming of newly-discovered alleles presents the main problem. This has been discussed by Cohen & Shreffler (1961) with particular reference to the mouse transferrin locus, but their remarks are applicable to other species and other multiple allelomorphic systems. Cohen & Shreffler state, 'If new electrophoretic types of transferrin are found these should be designated alphabetically in order of discovery. If the same letter should happen to be assigned to two different types these might be distinguished by prefacing the duplicated letter with another,

designating for example the laboratory which reported it.' This system has been used in naming the newly discovered alleles reported in this paper.

The locus symbol for the polymorphic β-globulins was originally 'β'. Following the discovery (Smithies & Hiller, 1959; Giblett, Hickman & Smithies, 1959) that these β-globulins represented the iron-binding protein transferrin or siderophilin in human and cattle serum these authors adopted the locus symbol 'Tf' as more descriptive than 'β'. The locus symbol 'Tf' has been adopted in this publication also, following the observation that both proteins of the β-globulin zone pair in sheep bind iron.

3. RESULTS

(i) *Newly-detected alleles*

A survey of Merino serum samples showed the presence of a considerable number of phenotypes which had not been seen in sheep serum samples examined in England (Ashton, 1958*a*). Fourteen out of the fifteen possible types from five alleles had previously been seen and the alleles had been defined as sheep β^A, β^B, β^C, β^D and β^E according to decreasing order of mobility of the transferrin zone pairs in starch-gel. The fifteenth type *TfEE* has now been found (Sellers & Mitchell, personal communication).

Serum samples of known genotype, originating from the laboratory where the sheep transferrin types were first defined were compared with the Merino serum samples listed above. A number of previously unrecognized phenotypes were found. By comparison with the reference samples, and knowing that each allele in sheep produces a zone pair consisting of a faster moderately staining and a slower more intensely staining zone, it was possible to identify seven additional sheep transferrin alleles as follows:

(*a*) Two alleles producing zone pairs migrating more rapidly than that from Tf^A. These have been called TF^F and Tf^G, Tf^F producing the fastest transferrin zone pair found so far in sheep sera.

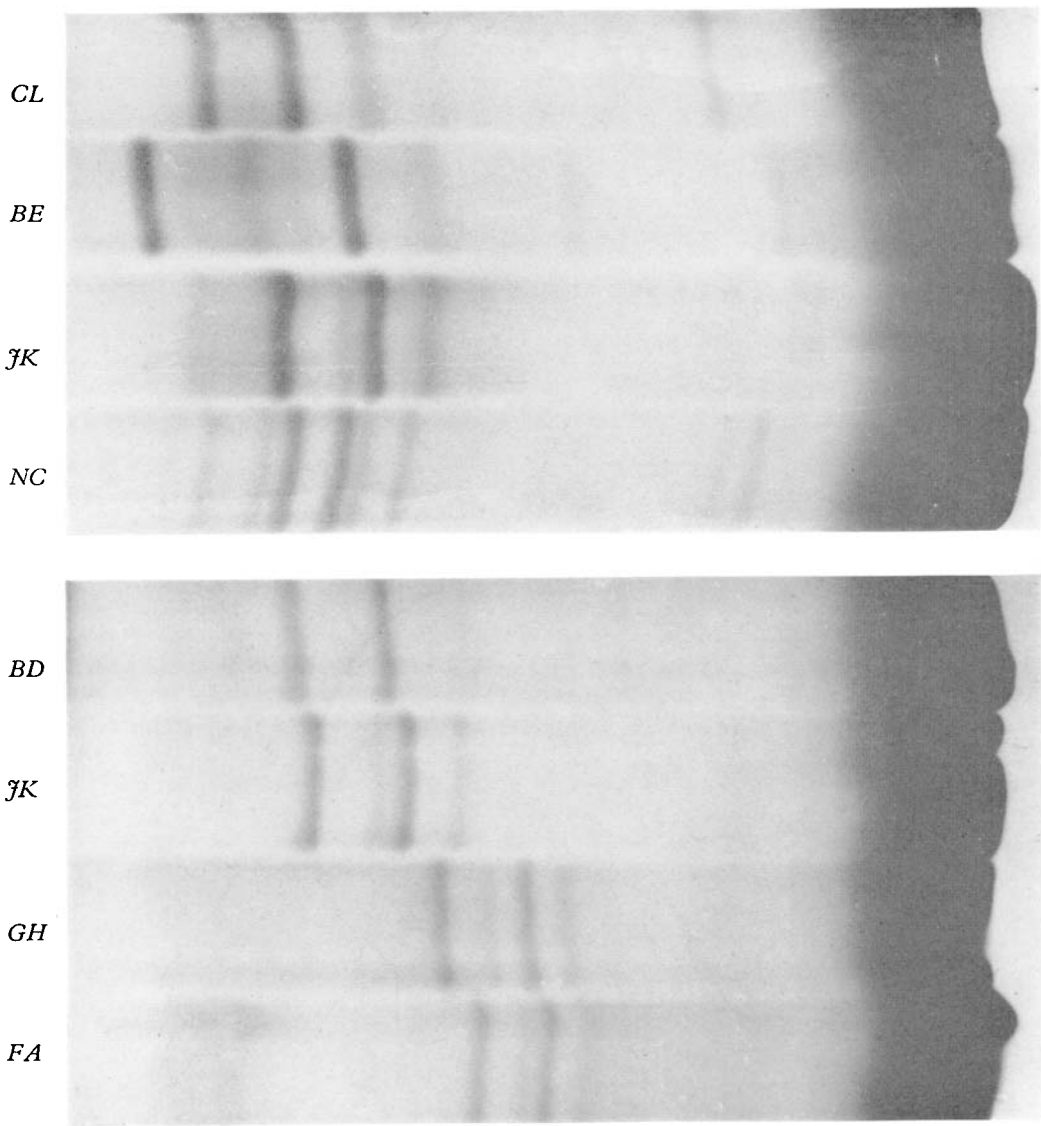
(*b*) Two alleles producing zone pairs intermediate in mobility between those produced by Tf^A and Tf^B. These alleles have been called Tf^H and Tf^J, Tf^H producing the faster zone pair.

(*c*) An allele producing a zone pair between those produced by Tf^C and Tf^D. This allele has been called Tf^K.

(*d*) An allele producing a zone pair between those produced by Tf^D and Tf^E. which has been called Tf^L.

(*e*) An allele producing a zone pair between those produced by Tf^B and Tf^C. This allele has been called Tf^N.

The alleles have been coded in the order in which they were recognized, and not in order of decreasing mobility. The relative position of the twelve defined sheep transferrin alleles are shown in Fig. 1 and examples of phenotypes produced by these alleles are shown in Plate I.



Starch gel electrophoresis of transferrin *A* in sheep sera. 17 V/cm for 21 hours. 90% 0·0033 M citric acid, 0·025 M tris (hydroxymethyl) aminomethane, 10% 0·02 M lithium hydroxide, 0·076 M boric acid in gel. 0·1 M lithium hydroxide, 0·38 M boric acid in electrode vessels.

(*Facing page* 242)

https://doi.org/10.1017/S001667230000358X Published online by Cambridge University Press

(*a*) Tf^F *and* Tf^G

Zone pairs migrating more rapidly than that produced by Tf^A occurred frequently in the first samples examined. Subsequently it was realized that two zone pairs were present, with only slightly different mobilities. The faster zone pair was defined as due to the allele Tf^F which is quite common in Merinos, and the slower to Tf^G which is rather infrequent. It was found advisable to re-run all samples with, Tf^F or Tf^G represented to verify the phenotype.

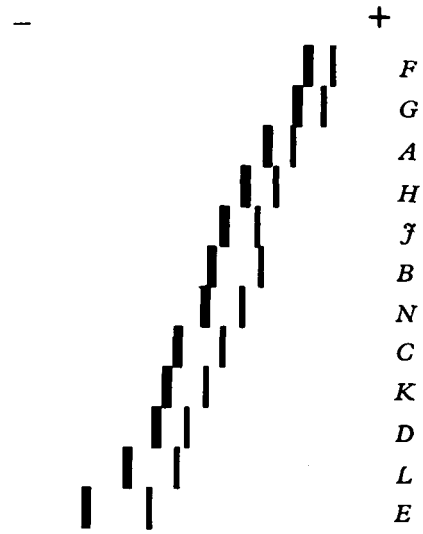


Fig. 1. Relative mobility of transferrin types in sheep sera by starch gel electrophoresis. Electrophoretic conditions as described in Plate 1.

(*b*) Tf^H *and* Tf^J

The zone pairs produced by Tf^H and Tf^J are well-resolved from each other and from Tf^A and Tf^B. Tf^H and Tf^J alleles are frequent in Merinos.

(*c*) Tf^K, Tf^L, Tf^N

Only a small number of phenotypes produced by these newly discovered alleles were found. Several hundred samples were examined before a Tf^N heterozygote was found. The homozygote, Tf^L/Tf^L has been found, but not the homozygotes Tf^K/Tf^K or Tf^N/Tf^N.

The zone pairs produced by Tf^K, Tf^L, and Tf^N are readily distinguishable from the zone pairs preceding or succeeding them in starch gel.

(*d*) Tf^A *and* Tf^C

Tf^A was fairly frequent and Tf^C infrequent in the serum samples examined.

(*e*) Tf^B, Tf^D, *and* Tf^E

Zone pairs due to these alleles were not found in the Merino serum samples examined in this survey.

https://doi.org/10.1017/S001667230000358X Published online by Cambridge University Press

(ii) TfDD *reference samples*

The zone pairs due to Tf^D in the heterozygous English reference sera $TfAD$, $TfBD$, $TfCD$ and $TfDE$ had the same electrophoretic mobility. Using these sera Tf^K and Tf^L were defined as alleles producing zone pairs migrating faster (Tf^K) and slower (Tf^L) than that produced by Tf^D.

Two examples of nominal $TfDD$ reference sera were also available. On examination in the discontinuous tris buffer system these samples proved to be heterozygotes, one being $TfDL$ and the other $TfKL$. Apart from illustrating the markedly improved resolution achievable with the discontinuous buffer system, these two samples show that Tf^K and Tf^L occur in British breeds of sheep, probably with reasonable frequency.

(ii) *Deterioration of reference samples*

The definition of new alleles depends on the comparison of a serum showing a suspected new phenotype with reference sera. It is important therefore that the reference sera should not have changed in electrophoretic mobility prior to the comparison.

The effect of heat (warming in a water bath at 50° C) and bacterial contamination (exposure to laboratory atmosphere) on the mobility of transferrin zone pairs of several sera of known phenotype was examined. In each case both treatments eventually produced a phenotype with double the number of zone pairs originally present. In every case the newly formed zone pairs were slower in mobility than the original. On prolonged treatment the zones became diffuse and eventually unrecognizable.

This behaviour is similar to that found with red deer phenotypes by Lowe & McDougall (1961). It may be due to the splitting-off of sialic acid from the transferrins by neuraminidase (Poulik, 1959; Blumberg & Warren, 1961).

None of the English reference sera, which have been stored continuously at −17° C. for four years, has shown any signs of such deterioration.

(iv) *Mating results*

No exceptions have been found, in a variety of animal species examined, to the hypothesis that transferrin polymorphism in each species is controlled by a multiple autosomal allelomorphic system exhibiting co-dominance. A relatively small number of mating results in English sheep (Ashton 1958*a*) fitted such a hypothesis. The distributions of 177 offspring from Merino ewes and rams, involving the transferrin alleles F, G, A, H, J and K, were in accord with the hypothesis.

Extensive mating data are being collected, in co-operation with Miss H. N. Turner and Mr G. H. S. Dolling, to test for fertility effects similar to those found in cattle (Ashton, 1961; Ashton & Fallon, 1962) and will be reported later.

(*v*) *Distribution of alleles in flocks examined*

Tf^F, Tf^A, Tf^H, Tf^J and Tf^K were present in all four flocks. Tf^G was present in only two of the four flocks, namely in those from Western Central Queensland and

the F. D. McMaster Field Station. Tf^L and Tf^N were found in low frequency, and only in the flock at the National Field Station. Out of 1356 sheep sampled there were two Tf^L homozyogtes and seventeen Tf^L heterozygotes. No Tf^N homogyzotes were found, but there were thirteen Tf^N heterozygotes. The National Field Station flock was the only flock in which Tf^C was found. There were five Tf^C homozygotes and thirty-eight heterozygotes. It is not yet known if Tf^C was present because of the Border-Leicester ancestry of some of the sheep sampled.

Because the National Field Station flock has been subjected to strong selection pressures for different factors, it is unlikely that gene frequencies found for the 1356 sheep sampled would be representative of Meirnos in general. The gene frequencies for the other three flocks are shown in Table 1, but no attempt was made to ensure that the samples taken were representative of the flocks in the area from which they were drawn.

Table 1. *Transferrin gene frequencies and standard errors for various sheep populations studied*

Population	No. of animals	Tf^F	Tf^G	Tf^A	Tf^H	Tf^J	Tf^K
Tasmanian fine-woolled Merinos	210	0·012 ± 0·005	—	0·314 ± 0·023	0·279 ± 0·019	0·371 ± 0·028	0·024 ± 0·007
Peppin Merinos from Central Western Queensland	298	0·181 ± 0·016	0·059 ± 0·010	0·149 ± 0·015	0·200 ± 0·016	0·391 ± 0·020	0·020 ± 0·006
Peppin Merinos from Badgery's Creek, N.S.W.	71*	0·112 ± 0·037	0·084 ± 0·033	0·075 ± 0·031	0·187 ± 0·046	0·458 ± 0·059	0·084 ± 0·033

* Excluding inbred animals, and counting maternally bestowed genes only in the group of offspring from ewes mated to inbred rams.

4. DISCUSSION

The recognition of a further seven transferring alleles in sheep, brings the number of described alleles to twelve. Sellers & Mitchell (personal communication) have found a phenotype with a zone pair migrating more slowly than that produced by Tf^E in a sample from a Derbyshire Gritstone sheep. These authors have also seen phenotypes with zone pairs migrating faster than that produced by Tf^A. It seems probable that these represent Tf^F or Tf^G or both. Direct comparison between samples will be necessary to define similarities or differences in mobility.

There are clearly marked breed differences in the type of genes represented, and the frequency of these genes, between the British breeds examined previously (Kerry Hill, Welsh Mountain, and Lincoln) and the Australian Merino. Also there may be within-breed differences between Tasmanian fine-woolled and Queensland Peppin Merinos (Table 1). Further and more strictly controlled sampling will be necessary to establish between- and within-breed gene frequencies.

There are probably at least thirteen transferrin alleles in English and Merino sheep. Examination of other breeds from other parts of the world may well reveal further alleles. Polymorphism is considered to be a means whereby a species is able to adapt to varying environments and ecological situations. As such polymorphism, and particularly biochemical polymorphism, is worthy of study by animal breeders concerned with choosing the right type of animal for a given location. The widespread geographical occurrence of sheep may be a reflection of their high level of biochemical polymorphism, among which the serum transferrins may be important.

Two ways in which transferrin polymorphism may influence the adaptability of sheep breeds may be inferred from results obtained in cattle. It has been shown that milk yield of dairy cattle is correlated with transferrin type (Ashton, 1960*b*; Ashton, Fallon & Sutherland, 1962). If a similar association occurs in sheep the growth rate of suckling lambs, and hence their chance of survival, wool production, etc., may be partly related to maternal transferrin type. It must be emphasised, however, that such effects would be detected only by determining the average values (milk yield, birth-weaning gain, etc.) of a large random group of animals, and would have no value in predicting, for example, the milk yield of an individual.

It has also been shown that parental transferrin type affects the fertility of artificially inseminated dairy cattle (Ashton, 1961; Ashton & Fallon, 1962). Thus matings between homozygotes proved very significantly more fertile than matings involving heterozygotes. If the parental β-globulin type in sheep is found to influence fertility, as in cattle, then this might have practical significance in increasing conception rates in artificial insemination.

5. SUMMARY

Serum samples from 1963 Merino sheep were examined for serum transferrin type. Two of the five transferrin alleles previously described in British breeds of sheep, viz. Tf^A and Tf^C, were found, but Tf^B, Tf^D and Tf^E were absent. Evidence for seven further transferrin alleles was obtained. These alleles were coded Tf^F, Tf^G, Tf^H, Tf^J, Tf^N, Tf^K and Tf^L in decreasing order of mobility of the zones they produce in starch gel.

Gene frequency data is presented for the populations studied.

Blood samples and records from Tasmania were kindly supplied by Mr P. M. Houlahan of the Department of Agriculture, Launceston, and from the McMaster Field Station, Badgery's Creek, N.S.W., by Mr R. Hayman, Officer-in-Charge. We are grateful to Miss H. N. Turner, Mr G. H. S. Dolling, and Professor J. V. Evans for making available blood serum samples from the National Field Station, 'Gilruth Plains', Cunnamulla, Queensland, and allowing us to present gene frequency data from this co-operative project; and to Dr G. R. Moule for inviting us to examine serum samples from sheep in one of his experiments in Western Central Queensland.

REFERENCES

Ashton, G. C. (1957). Serum protein differences in cattle by starch gel electrophoresis. *Nature, Lond.*, **180**, 917–919.

Ashton, G. C. (1958*a*). Further β-lgobulin phenotypes in sheep. *Nature, Lond.*, **182**, 1101–1102.

ASHTON, G. C. (1958*b*). Serum protein variations in horses. *Nature, Lond.*, **182,** 1029–1030.
ASHTON, G. C. (1960*a*). Thread protein and β-globulin polymorphism in the serum proteins of pigs. *Nature, Lond.*, **186,** 991–992.
ASHTON, G. C. (1960*b*). β-globulin polymorphism and economic factors in dairy cattle. *J. agric. Sci.* **54,** 321–328.
ASHTON, G. C. (1961). β-globulin type and fertility in artificially bred dairy cattle. *J. Reprod. Fert.* **2,** 117–129.
ASHTON, G. C. & BRADEN, A. W. H. (1961). Serum β-globulin polymorphism in mice. *Aust. J. biol. Sci.* **14,** 248–253.
ASHTON, G. C. & FALLON, G. R. (1962). β-globulin type fertility, and embryonic mortality in cattle. *J. Reprod. Fert.* **3,** 93–104.
ASHTON, G. C., FALLON, G. R. & SUTHERLAND, D. O. (1962). β-globulin (transferrin) type and milk and butterfat production in dairy cows. *J. agric. Sci.* (In the press.)
ASHTON, G. C. & MCDOUGALL, E. I. (1958). β-globulin polymorphism in cattle, sheep and goats. *Nature, Lond.*, **183,** 945–946.
BLUMBERG, B. S. (1960). Biochemical polymorphisms in animals: Haptoglobins and transferrins. *Proc. Soc. exp. Biol. N.Y.* **104,** 25–28.
BLUMBERG, B. S. & WARREN, L. (1961). *Biochem. biophys. Acta*, **50,** 90.
BOYER, S. H. & YOUNG, W. J. (1960). β-globulin polymorphism in chimpanzees. *Nature, Lond.*, **187,** 1035–1036.
BUETTNER-JANUSCH, J. (1961). Transferrin differences in chimpanzee sera. *Nature, Lond.*, **192,** 632–633.
COHEN, B. L. (1960). Genetics of plasma transferrin in the mouse. *Genet. Res.* **1,** 431–438.
COHEN, B. L. & SHREFFLER, D. C. (1961). A revised nomenclature for the mouse transferrin locus. *Genet. Res.* **2,** 306–308.
FERGUSON, K. A. & WALLACE, A. L. C. (1961). Starch-gel electrophoresis of anterior pituitary hormones. *Nature, Lond.*, **190,** 629–630.
GAHNE, B. & RENDEL, J. (1961). Blood and serum groups in reindeer compared with those in cattle. *Nature, Lond.*, **192,** 529–530.
GIBLETT, E. R., HICKMAN, C. G. & SMITHIES, O. (1959). Serum transferrins. *Nature, Lond.*, **183,** 1589–1590.
GOODMAN, M. & POULIK, E. (1961). Serum transferrins in the genus *Macaca*: Species distribution of nineteen phenotypes. *Nature, Lond.*, **191,** 1407–1408.
HICKMAN, C. G. & SMITHIES, O. (1957). Evidence for inherited differences in the serum proteins of cattle. *Proc. gen. Soc. Can.* **2,** 39.
KRISTJANSSON, F. K. (1960). Inheritance of a serum protein in swine. *Science*, **131,** 1681.
LAI, L. Y. C. & KIRK, R. L. (1960). β-globulin variants in two species of monkeys. *Nature, Lond.*, **188,** 673–674.
LOWE, V. A. W. & MCDOUGALL, E. I. (1961). Serum β-globulin types in red deer and other species and their stability in the presence of bacteria. *Nature, Lond.*, **192,** 983–984.
MILLSON, G. C. & PATTISON, I. H. (1961). β-globulin polymorphism in goats. *Vet. Rec.* **73,** 256.
PATRAS, B. & STONE, W. H. (1961). Partial purification of cattle serum transferrin using rivanol. *Proc. Soc. exp. Biol., N.Y.*, **107,** 861–864.
POULIK, M. D. (1957). Starch gel electrophoresis in a discontinuous system of buffers. *Nature, Lond.*, **180,** 1477–1479.
POULIK, M. D. (1959). Starch gel immuno-electrophoresis. *J. Immunol.* **82,** 502–515.
SHREFFLER, D. C. (1960). Genetic control of serum transferrin type in mice. *Proc. nat. Acad. Sci., Wash.*, **46,** 1378–1384.
SMITHIES, O. (1955). Zone electrophoresis in starch gels: group variations in the serum proteins of normal human adults. *Biochem. J.* **61,** 629–641.
SMITHIES, O. (1957). Variants in human serum β-globulins. *Nature, Lond.*, **180,** 1482–1483.
SMITHIES, O. & HILLER, O. (1959). The genetic control of transferrins in humans. *Biochem. J.* **72,** 121–126.