Papers and Originals

TISSUE TRANSPLANTATION: A NEW APPROACH TO THE "TYPING" PROBLEM

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When the transplantation of tissue from one individual to another is to be attempted in clinical practice the surgeon may sometimes be able to choose between a number of possible donors. The "typing problem" is the problem of how best to exercise this choice, in so far as it depends on purely immunological considerations—that is, of how to choose the donor whose tissues are least foreign to the future recipient. Such a choice may be crucially important, for experience with laboratory animals has shown that when the transplantation of tissues is opposed only by weak immunological barriers it is a matter of no great difficulty to induce a state of immunological tolerance, even in adult life (Martinez, Shapiro, Kelman, Onstad, and Good, 1960; McKhann, 1962; Medawar, 1963).

The test of compatibility we propose here is founded on the observation that the homograft reaction in guinea-pigs and human beings can be made to display itself as a cutaneous inflammatory reaction of delayed onset, essentially similar to a tuberculin reaction (Brent, Brown, and Medawar, 1958, 1962; Merrill, Friedman, Wilson, and Marshall, 1961). Let R be the intended recipient of a homograft and D_1, D_2, D_3, etc., a panel of possible donors. Lymphocytes are extracted by differential sedimentation from the defibrinated blood of R and injected intradermally into each member of the panel of donors, whereupon they give rise to small but otherwise typical "delayed reactions" of unequal intensities. Experiments on guinea-pigs have shown that the order of the strengths of the inflammatory reactions excited by R blood lymphocytes in the skins of D_1, D_2, D_3, etc., is almost exactly correlated with the order of breakdown of skin grafts subsequently transplanted from D_1, D_2, D_3, etc., to R. The weakest reactor is the donor of the most long-lived homograft and therefore the donor of choice.

The existence of this correlation makes it very likely that the inflammatory reaction provoked by R lymphocytes in D skin is, in fact, an R-*versus*-D homograft reaction, but one that is being played, so to speak, as an away match—that is, in the D guinea-pigs instead of in R. That R lymphocytes should give rise to such a reaction in D skin is entirely in keeping with our knowledge of "graft against host" reactions and of the immunological competence of lymphocytes (Billingham and Brent, 1957, 1959; Simonsen, 1957; Gowans, Gesner, and McGregor, 1961). Cock and Simonsen (1958) have already shown that, in chicks, allogeneic blood leucocytes can cause local swellings after injection into the skin, and Terasaki's (1959) more detailed analysis of the phenomenon showed that the cells responsible for them were almost certainly lymphocytes.

Although the method we propose has been tested only on guinea-pigs, we have no reason to doubt its applicability to human beings. Indeed, it seems likely that reactions of the kind we describe have already been seen in human beings (Walzer, Bowman, and Stroyman, 1957; H. S. Lawrence, personal communication). It is true that Merrill *et al.* (1961) describe as "negative" the reactions raised by the intradermal injection of normal allogeneic blood lymphocytes in human beings, but for their special purposes inflammatory areas less than 10 mm. in diameter were regarded as negative, and many reactions must therefore have been excluded which would have been classified as positive by us.

Subjects and Reagents

The guinea-pigs belonged to two strains: (*a*) the Hartley strain, a closed colony of albinos with a range of antigenic variation that made them a realistic model of a human population; and (*b*) the Heston strain of strictly inbred and virtually syngeneic tricoloured guinea-pigs derived remotely from Sewall Wright's Strain 2. The tests were carried out mainly during the winter months, and we found it advisable to enrich the animals' diet with a few milligrams of ascorbic acid given daily by mouth.

The accelerated sedimentation of erythrocytes was brought about by mixing defibrinated blood with not less than an equal volume of a neutral sterile 3% solution of salt-free high-molecular-weight (~200,000) dextran in unbuffered Ringer's solution. In earlier experiments, on the recommendation of Professor R. Ceppellini, we used a neutral sterile 3% solution of gelatin in unbuffered Ringer's solution, but we prefer dextran solution because it is fluid at room temperature. For human blood the use of phytohaemagglutinin might well be investigated, but there is no reason to think that the "activation" of lymphocytes brought about by certain samples (Nowell, 1960) would be advantageous.

It is unconditionally necessary that all media should be completely non-toxic, a requirement that disqualifies agents such as "polybrene" (hexadimethrine bromide). All gelatin and dextran solutions, after neutralization, were tested for toxicity by mixing them with an equal volume of freshly withdrawn washings from the peritoneal cavities of normal mice (2 ml. Ringer-phosphate per mouse), and then incubating the mixture in culture vessels for 48 hours to make sure that the reagents had no perceptibly harmful effect upon the peritoneal macrophages spread out on the glass surface.

Detailed Procedure

We shall describe our most complete single experiment (experiment 20, Table II) in detail and add notes on actual or possible departures from the procedure described. In this experiment all the guinea-pigs belonged to the Hartley strain (see note *a*). All manipulations except the incubations at 37° C. were carried out at room temperature.

Seventeen millilitres of blood (see note *b*) were withdrawn from the heart of the future recipient, R, transferred to a 50-ml. centrifuge tube, and defibrinated (see note *c*) by twirling in it a glass rod bent into zigzag angles until fibrin fibres were no longer formed. A roughly equal volume of dextran solution was mixed with the defibrinated blood and the mixture incubated in the upright position for 30

minutes (see note *d*), during which it separated into a supernatant layer containing most of the lymphocytes and a compact sediment (see note *e*) containing most of the red cells.

The supernatant layer was spun for 10 minutes at a low speed (~1,500 r.p.m., radius 15–20 cm.), producing a cell-free top layer and a sediment rich in lymphocytes but still too heavily contaminated by red cells for immediate use. This second sediment was therefore resuspended in about 1 ml. of the fluid overlying it and incubated for a further 45 minutes. Most of the red cells settled out to form a third sediment, which was discarded; the top layer was spun for a second time as above and the cellular deposit finally resuspended in 1.2 ml. Ringer-phosphate (see note *f*).

In experiment 20 the yield of nucleated cells per ml. defibrinated blood was 3.8 million, of which 12.8% were granulocytes (an unusually high figure: see Tables II and III) and the remainder lymphocytes. Red cells were present in about the same number as white cells—an acceptably low degree of contamination.

The cell donor R (itself a female) and five small Hartley females representing the donor panel (D_1, D_2, D_3, D_4, D_5) each now received two intradermal injections of 0.10 ml.=4.4 million lymphocytes into the skin of the flank (see note *g*). The reactions they gave rise to were inspected at 24 hours and thereafter daily. The reactions were of course small. The system of scoring adopted—for which no special merit is claimed—followed that of Brent *et al.* (1962); it took account of the diameter of the area of inflammation (sometimes as great as 7 or 8 mm.) and of its redness, hardness, and degree of swelling (see note *h*).

Table I shows the scores allotted during the first four days in experiment 20. In general only the scores at 24 and 48 hours can be used to forecast the behaviour of D skin grafts upon R. We have found repeatedly that from four days onwards the order of intensity may change completely: some reactions rise, others fade away. The sharp rise in the strength of some reactions suggests that a new phenomenon has supervened—almost certainly a homograft reaction of D against R.

TABLE I.—*Experiment 20: Daily Reaction Scores After Intradermal Injection of Normal Blood Lymphocytes from a Future Homograft Recipient (R) Into a Panel of Five Donors (D_1–D_5). (Hartley Strain Guinea-pigs)*

Day	Scores (Two Injection Sites per Guinea-pig)						Order of Intensity
	D_1	D_2	D_3	D_4	D_5	R	
1	++, ++	±, ±	+±, +	+, +	+, +	0	1>3≥5≥4>2
2	+±, +±	0, 0	+±, +	±, ±	<+, <+	0	1>3>5>4>2
3	—	—	—	—	—	0	1>3=5>4≥2
4	+±, +±	+, +±	++, ++	0, 0	++, ++	0	3>5>1=2>4

Note change of order on fourth day (see text). Detailed scores were not given on the third day.

After 12 days, ear-skin grafts from each of the five donors were transplanted simultaneously to R by the technique of Billingham and Medawar (1951). The plaster jacket protecting the grafts was removed on the seventh day and grafts were inspected daily thereafter until the completion of breakdown (see note *i*).

The survival times of the grafts, taken to the nearest day, were as follows: D_1, 13 days; D_3, 13 days; D_5, 15 days; D_4, 18 days; D_2, 20 days, giving a final order $1 \geqslant 3 > 5 > 4 > 2$, graft D_1 being ranked just above D_3 because it had the higher degree of survival at 12 days. Thus the order of breakdown fulfilled very accurately the prediction based upon the lymphocyte transfer test.

Notes on the Above

(*a*) In our experience the intradermal injection of 5 million normal *rabbit* blood lymphocytes into normal rabbits of the same or other breeds raises no outwardly visible reaction during the period in which a graft-against-host reaction might be supposed to be in progress. (A direct homograft reaction against the grafted cells may become apparent a few days later.) This is rather surprising, for the "transfer reaction" using presensitized blood lymphocytes is rather violent in the rabbit (Brent *et al.*, 1958, 1962; see also Mannick and Egdahl, 1962; Dvorak, Kosunen, and Waksman, 1963).

(*b*) *Blood* lymphocytes must be used (though thoracic duct lymphocytes should do just as well). Cells expressed from normal lymph nodes provoke virtually no reaction at dosages of 5 million or less per injection site, and are therefore unsuitable. Brent *et al.* (1962) had already observed without comment that normal allogeneic blood leucocytes can give rise to inflammatory reactions after intradermal injection: in 13 out of their 30 trials the reaction scores ranged from + to ++. On the other hand, repeated trials have shown that normal undamaged blood lymphocytes, like lymph-node cells, raise no reaction when injected into their own donor or into guinea-pigs syngeneic with their donor.

(*c*) Defibrination removes all platelets and many granulocytes. The proportion of lymphocytes tends to rise during manipulation, for granulocytes, unlike lymphocytes, tend to stick together and settle out.

(*d*) These times can be varied within quite wide limits.

(*e*) In this experiment, as in all others with guinea-pigs, the first sediment was re-extracted to get the maximal yield of cells from the comparatively small volume of blood obtainable from a single animal. With human blood, available in larger quantities, this second cycle of extraction should not be necessary; the first sediment can be discarded and the whole procedure can take the simple form outlined in the text.

(*f*) Cells awaiting use should not be kept *below* room temperature. The small quantities of serum and dextran remaining in the final suspension are probably advantageous. Serum itself raises no reaction, and Battisto's (1960, 1963) natural serum factor had no opportunity to interfere with our readings. (Battisto found that about 95% of the Rockefeller colony of Hartley guinea-pigs possess serum factor and therefore do not react to it; Strain II, the ancestor of our "Heston" strain, lacks the factor, so that serum transfers in the direction Heston→Hartley should in any case be innocuous (see Table III).)

(*g*) In guinea-pigs we have had satisfactory results with lymphocyte dosages as low as 2.5 million per injection site, though we usually aimed at doses of about 4 million. Titrations of *presensitized* blood lymphocytes in guinea-pigs show that perceptibly positive reactions may be raised by the intradermal injection of so few as 0.05 million lymphocytes.

(*h*) Experience has shown that the *absolute* value of the score given to the reaction raised by R lymphocytes in D cannot be accurately translated into a figure representing the survival time of D skin on R. It is the *relative* score or rank within any one experiment that matters. The scores are comparable only when they refer to injections made into anatomically comparable sites, for within any one individual the variation from one anatomical site to another may be as great as the variation from one individual to another.

(*i*) The length of the interval between the lymphocyte test and the skin-grafting is of course entirely optional. When ear-skin grafts are used (their thinness makes for ease and speed of healing) it is sometimes difficult to determine the end-point of epithelial survival. In one experiment (experiment 29A,B, Table II) creeping replacement by the hosts' epithelium was so insidious that no satisfactory end-point could be decided upon in spite of careful daily inspections.

Results

Two series of experiments were done. In the first (summarized in Table II) donors and recipients both

TABLE II.—*Typing Experiments with Hartley Strain Guinea-pigs*

Expt. No.	Lymphocyte Yield per ml. Defibrinated Blood ($\times 10^6$)	Granulocytes (%)	Lymphocyte Injection Dose ($\times 10^6$)	R Lymphocytes in D Guinea-pigs: Order and Strength of Reactions	D Skin on R Guinea-pigs: Order of Breakdown and Survival Times in Days	Notes
19	3·6	1·3	4·5	*1*⩾*3*>*4*=*2* (++ to ± at 24 hrs.)	—	*a*
20	3·3	12·8	4·4	*1*>*3*>*5*>*4*>*2* (++ to ±)	1⩾3>5>4>2 (13 to 20)	*a, b*
21	1·4	<2	3·4	3>5>2>4>1 (+± to 0 at 24 hrs.)	—	*a, c*
23	2·7	—	4·5	*4*⩾*1*>*3*⩾*2* (++ to ±)	4>1>2>3 (10 to 12½)	*a*
24	4·4	12·8	5·3	*1*=*4*>*2*⩾*3* (+± to +)	1=4>2>3 (11 to 18)	*a*
25	2·2	1·3	4·5	4⩾2⩾3>1 (++ to ±)	4⩾3 (9 to 40)	*a, d*
26	2·8	<1	5·5	*2*⩾*1*⩾*4*⩾*3* (++ to ±)	—	*a, c*
29A	2·5	4·1	3·6	8⩾7⩾5⩾6=4⩾3>2⩾1 (+++ to ±)	—	*e*
29B	3·2	5·4	5·4	*2*⩾*4*>5=6=8=*3*⩾*1*>7 (+++ to ±)	—	
32	1·4	3·0	2·5	*3*⩾*1*⩾*2*>*4* (+ to ±)	3>4 (12½ to 14½)	*f*
33	5·5	7·8	5·0	*1*>*2*=*4*>*3*	—	*c*

NOTES.—Italicized entries in column 5 refer to *female* guinea-pigs. Dextran sedimentation in all experiments. *a*, control injection (R→R) negative. *b*, see Table I. *c*, R pig died: the high mortality in this group is presumably associated with the withdrawal of blood for the typing test prior to grafting. *d*, R pig pregnant. *e*, see text, note (*i*); the same panel of recipients was used in expts. 29A and 29B. *f*, body-skin (not ear-skin) grafts.

TABLE III.—*Typing Experiments with Heston Strain Homograft Recipients (R) and Hartley Strain Homograft Donors (D)*

Expt. No.	Lymphocyte Yield per ml. Defibrinated Blood ($\times 10^6$)	Granulocytes (%)	Lymphocyte Injection Dose ($\times 10^6$)	R Lymphocytes in D Guinea-pigs: Order and Strength of Reactions	D Skin on R Guinea-pigs: Order of Breakdown and Survival Time in Days	Notes
13	1·1	13	4·8	1>2>*3*=*4* (++± to +±)	1>2>3>4 (<7 to 9)	*a*
14	0·9	<1	3·4	2⩾1>*3*⩾*4* (++± to +±)	2>1>4⩾3 (8½ to 10½)	*a, c*
15	1·0	6	4·4	*4*⩾1=*3*>2 (++ to +±)	—	*a, c, d, e*
17	1·0	5	5·0	2>1>*4*>*3* (++± to +)	1>4>3 (8 to 9)	*a, c, d*
18	1·2	10	4·8	1=2=*3*>*4* (+± to +)	1=2=3>4 (7 to 8)	*a, c*
22A 22B	0·8	<2	2·7	*2*⩾*3*⩾*4*⩾*1* (+++ to ± at 24 hr.) 6⩾7⩾5 (+± to + at 24 hr.)	2=4>3>1 (7½ to 11) 6=7>5 (9 to 10)	*b*
27A 27B	1·3	<1	3·1	*2*>*1*>*3*=*4* (+++ to +) 1=2=3=4 (+±)	3=4 (8) 1=2=3=4 (7)	*b*
36	0·9	<1	3·6	6⩾*2*⩾*1*=*4*=5>*3* (+++ to +)	6>3 (8 to 11)	*b*

NOTES.—Italicized entries in column 5 refer to *female* guinea-pigs. *a*, gelatin used for sedimentation. *b*, dextran used for sedimentation. *c*, control injections (Heston→Heston) negative. *d*, reaction scores for same number of lymph-node cells, < +. *e*, grafts lost by infection.

belonged to the Hartley strain; in the second series (Table III) the R guinea-pigs belonged to the Heston strain and the D guinea-pigs to the Hartley strain. The use of donors and recipients belonging to unrelated strains led to generally stronger lymphocyte transfer reactions and to shorter and less sharply differentiated graft-survival times (though a remarkably fine degree of discrimination was still possible).

The correspondence between the lymphocyte transfer scores and the intensity of the skin-homograft reactions was very close, though there are unimportant departures from expectation in experiments 14, 22, and 23. Experiments 29A and 29B were spoiled by the inability to determine survival end-points mentioned in note (*i*) above, but they show that lymphocytes belonging to two different populations can raise reactions of different intensities in the same subject, for the same panel of guinea-pigs was used in both tests. In theory the lymphocyte-transfer reaction and the corresponding skin-homograft reaction are merely two different manifestations of the same phenomenon, and the correlation between them should be not merely close but exact. That it was not so in all cases was probably due to chance variations in the anatomical site or depth of the intradermal injections and to physiological variations among the donor panel in their readiness to manifest the inflammatory component of the reactions.

It would be a great mistake to suppose that the differences in survival times revealed by skin-grafting are immunologically trivial because they are relatively small. In experiment 24 (Table II), for example, the most long-lived graft survived 18 days, the most short-lived 11 days. In terms of immunological reactivity the difference between them is profound: an 18-day survival time reveals an immunological opposition which should be easy to overcome by methods already under clinical trial.

Applicability to Human Beings

The method of matching or typing described above may be described as N.L.T. (normal lymphocyte transfer) typing for short. If our reasoning is correct, N.L.T. typing should be applicable to human beings, but in a clinical context certain special considerations should be borne in mind.

1. The danger of disseminating infectious disease by cellular transfer. Any test of the principle of N.L.T. typing that made use of human volunteers should be based on the use of blood from accredited blood donors.

2. While the test cannot affect the immunological reactivity of the patient in need of a homograft, the transfer of lymphocytes must at least temporarily sensitize the potential homograft donors. The intensity and duration of the sensitivity produced in human beings by two or three simultaneous inoculations of 5 million lymphocytes should be tested by the methods of Friedman, Retan, Marshall, Henry, and Merrill (1961).

3. The influence of "natural" antibodies of the ABO system on transferred lymphocytes must obviously be investigated. Dr. P. I. Terasaki tells us that, in his experience, powerful anti-A and anti-B sera do not agglutinate human lymphocytes in balanced salt solutions or dextran, and human blood leucocytes do not absorb antibodies from such sera even when used in concentrations of up to twentyfold that of red cells.

4. In clinical practice it would be advisable on all occasions to inject the lymphocyte donor with preparations of his own lymphocytes to make sure they raise no reaction of a non-immunological character.

5. At least some errors of ranking caused by differences among the donor panel of skin thickness, endocrinological status, vascular responsiveness, etc., must be regarded as inevitable. Only experience can decide whether or not the N.L.T. test is workable in clinical practice—either to provide a complete and reliable ranking of possible donors, as in

guinea-pigs, or to act in a more limited capacity—for example, as an exclusion test.

6. It can be assumed that the compatibility or incompatibility revealed by the N.L.T. test applies to organs other than skin.

7. The N.L.T. test is to be thought of as an *ad hoc* measure which may be useful until the adoption of methods analogous to those used in erythrocyte grouping provide a definitive solution of the typing problem.

General Considerations

The N.L.T. test is biologically interesting in two ways. It shows that blood lymphocytes are much more reactive than lymph-node cells—a discovery for which the work of Billingham, Silvers, and Wilson (1962) and of Gowans (1962) had already prepared us. Secondly, the inflammation raised in the N.L.T. test is almost the simplest imaginable outward sign that an immunological performance is in progress. The speed at which it develops is not surprising in the light of the evidence presented by Gowans (1962) and by Miller, Pierce, Martinez, and Good (1963). As a test of immunological competence—for which it is otherwise well suited—the N.L.T. reaction will of course be especially vulnerable to interference by the non-specific inflammation that may be provoked by the injection of damaged cells.

Summary

In guinea-pigs the intradermal injection of normal blood lymphocytes from the intended recipient of a homograft into each member of a panel of possible donors excites inflammatory reactions of delayed onset and unequal intensity. The intensity of this inflammatory response provides an almost exact forecast of the intensity of the reaction which a homograft from each donor will elicit after transplantation to the future recipient. Where choice can be exercised, this "normal lymphocyte transfer" (N.L.T.) test may therefore be made the basis of an *ad hoc* method for choosing the homograft donor most compatible with a given recipient. Subject to certain safeguards, the N.L.T. test should be applicable to human beings.

References

Battisto, J. R. (1960). *Nature (Lond.)*, **187**, 69.
—— (1963). Ibid., **198**, 598.
Billingham, R. E., and Brent, L. (1957). *Transplant. Bull.*, **4**, 67.
—— —— (1959). *Phil. Trans. B*, **242**, 439.
—— and Medawar, P. B. (1951). *J. exp. Biol.*, **28**, 385.
—— Silvers, W. K., and Wilson, D. B. (1962). *Lancet*, **1**, 512.
Brent, L., Brown, J. B., and Medawar, P. B. (1958). Ibid., **2**, 561.
—— —— —— (1962). *Proc. roy. Soc. B*, **156**, 187.
Cock, A. G., and Simonsen, M. (1958). *Immunology*, **1**, 103.
Dvorak, H. F., Kosunen, T. U., and Waksman, B. H. (1963). *Lab. Invest.*, **12**, 58.
Friedman, E. A., Retan, J. W., Marshall, D. C., Henry, L., and Merrill, J. P. (1961). *J. clin. Invest.*, **40**, 2162.
Gowans, J. L. (1962). *Ann. N.Y. Acad. Sci.*, **99**, 432.
—— Gesner, B. M., and McGregor, D. D. (1961). In *Biological Activity of the Leucocyte*, edited by G. E. W. Wolstenholme and M. O'Connor, p. 32. Churchill, London.
McKhann, C. F (1962). *J. Immunol.*, **88**, 500.
Mannick, J. A., and Egdahl, R. H. (1962). *Ann. Surg.*, **156**, 356.
Martinez, C., Shapiro, F., Kelman, H., Onstad, T., and Good, R. A. (1960). *Proc. Soc. exp. Biol. (N.Y.)*, **103**, 266.
Medawar, P. B. (1963). *Transplantation*, **1**, 21.
Merrill, J. P., Friedman, E. A., Wilson, R. E., and Marshall, D. C. (1961). *J. clin. Invest.*, **40**, 631.
Miller, J., Pierce, J. C., Martinez, C., and Good, R. A. (1963). *J. exp. Med.*, **117**, 863.
Nowell, P. C. (1960). *Cancer Res.*, **20**, 462.
Simonsen, M. (1957). *Acta path. microbiol. scand.*, **40**, 480.
Terasaki, P. I. (1959). *J. Embryol. exp. Morph.*, **7**, 394.
Walzer, M., Bowman, K. L., and Stroyman, S. (1957). *J. Allergy*, **28**, 206.

DIFFERENT PATTERNS OF HUMAN PORPHYRIA*

BY

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The delimitation of natural disease entities is one of the real difficulties in scientific medicine. We may accept the infectious conditions caused by a known agent such as tuberculosis and syphilis as "good" diseases. Others, like diabetes mellitus, are probably heterogeneous even if recent studies of heredity speak in favour of one chief factor. When we come to such old-fashioned concepts as chronic "myocarditis" everybody should be aware that these are no more than words without much meaning. There is, however, one group of diseases with a genuine right to be regarded as having a well-defined and identical origin. They are the inborn errors of metabolism. This is especially true of all cases occurring in the same family, where *one* mutation—that is, the same change in the structure of the D.N.A. molecule—must be at work. It may be dangerous, however, to postulate that all members of different families supposed to have the same disease are really suffering from the results of the same mutation—that is, that they are absolutely identical. Haemophilia is an excellent example of this. There are many learned calculations using impeccable statistical techniques of the mutation frequency of "haemophilia." We now know that haemophilia was diagnosed as one disease until clinical analysis (Koller, Krüsi, and Luchsinger, 1950) showed that there must exist two entirely different haemophilias: true=haemophilia A with deficiency of factor VIII, and Christmas disease=haemophilia B with deficiency of factor IX.

Recent work in Malmö has shown that a number of men suffering from von Willebrand's disease have probably earlier been regarded as haemophiliacs (Nilsson, Blombäck, and Blombäck, 1959). This group of haemorrhagic diseases is probably genetically still more heterogeneous. Several authors (Brinkhous and Graham, 1954; Nilsson, 1960) have pointed out the fact that there seem to be different degrees of haemophilia that always remain true to type—that is, they are inherited either as severe or as intermittent or as mild. Among the true haemophilia families there seem to exist three degrees of severity. This would mean that there are at least six different mutations. Von Willebrand's disease is inherited autosomally and obviously has quite a different mechanism. Linkage studies in this condition will ultimately show if this disease picture is always the result of mutations in the same locus or not.

It seems evident that all discussions regarding mutation frequencies are without scientific value so long as these fundamental clinical principles have not been recognized as basic.

When one of us (J. W.) became interested in the porphyrias, Günther (1911, 1922), who was a great authority,

*This is a slightly enlarged Litchfield Lecture given in Oxford in 1962 by one of us (J. W.).