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Haemorrhagic Disorders due to Functional Abnormalities of Platelets

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THE HAEMOSTATIC PROCESS

It has been known for many years that platelets play an important part in the arrest of haemorrhage from injured vessels, both by the formation of a mechanical plug and by contributing to the blood coagulation mechanism, and also that they are essential for subsequent normal clot retraction. During recent years there have been rapid advances in understanding the detailed roles of platelets in the haemostatic process and their interrelationships. Much of the work which has led to these advances has been stimulated by the observation that platelets, in blood or plasma, are rapidly aggregated by the addition of adenosine diphosphate (ADP) (Hellem, 1960; Gaarder *et al.*, 1961).

The sequence of events following injury to a small vessel is illustrated diagrammatically in Fig. 1. Within seconds of the blood coming into contact with the connective tissue in and around the vessel wall, particularly collagen, platelets adhere to connective tissue fibres, and this adhesion is immediately followed by release of ADP from these adherent platelets, and probably also from damaged connective tissue and red cells in the vicinity. This ADP not only causes further platelets to aggregate on top of those which have already adhered to the collagen, but results in the release of further ADP from them, thus bringing about the aggregation of more platelets by means of a chain reaction. Meanwhile, the plasma coagulation mechanism has been set in action, both through the intrinsic system, factor XII being activated by contact with collagen (Niewiarowski *et al.*, 1965), and also through the extrinsic system. Another effect of platelet aggregation is to make a platelet phospholipid component, platelet factor 3, available for the blood coagulation mechanism (Hardisty and Hutton, 1966), so that coagulation occurs more rapidly on and around the surface of the aggregated platelets. The thrombin formed in this way not only leads on to fibrin formation, but also causes further secondary changes in the aggregated platelets, as a result of which the platelet plug becomes consolidated and irreversible (Grette, 1962;

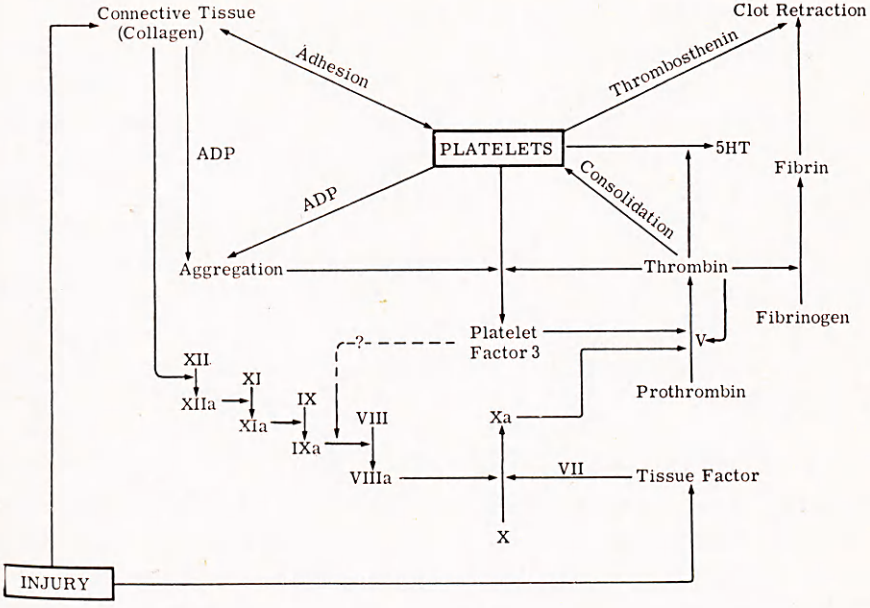


Fig. 1. The role of platelets in haemostasis

Spaet and Zucker, 1964). Platelets themselves contain fibrinogen (Nachman, 1965) and also have fibrinogen absorbed on to their surface, ensuring their intimate relation to the strands of fibrin as they are formed. Finally, it is the platelets that bring about clot retraction, by virtue of the contractile protein, thrombosthenin, that they contain (Bettex-Galland and Lüscher, 1961). Platelets also contribute to blood coagulation in two other ways: the anti-heparin factor of platelets (platelet factor 4) is released when platelets aggregate (Niewiarowski *et al.*, 1968; Youssef and Barkhan, 1968), and platelets also have an action like tissue extract in the extrinsic blood coagulation mechanism (Biggs *et al.*, 1968). Thus, it is clear that platelets play a central role in the haemostatic process and that the various aspects of this role are closely interdependent.

How then can this mechanism go wrong? We know that single deficiencies of plasma clotting factors will lead to a breakdown of the system; can we also identify a series of separate disorders of individual platelet functions, such as failure of adhesion or of aggregation, or of platelet factor 3 availability or clot retraction, each of which can bring about a specific bleeding disorder? This paper will show that, although a number of different functional disorders of platelets can now be recognised, they cannot be simply identified in this

way as defects of individual functions, largely because of the close interdependence between these various roles of platelets in haemostasis. Thus, any failure of platelet aggregation will result in defects of platelet factor 3 availability and platelet factor 4 release, and perhaps of clot retraction, and will also be associated with decreased adhesiveness to glass in most instances, as well as with a long bleeding time. Such defects of platelet aggregation, on the other hand, can result from different types of underlying platelet abnormality and some of these can in turn arise in a number of different clinical situations. The actual biochemical basis of these abnormalities has yet to be elucidated, as has the mechanism by which ADP normally brings about platelet aggregation and the release of further ADP and other active principles from the platelets.

THROMBASTHENIA (GLANZMANN'S DISEASE)

Fifty years ago Glanzmann (1918) described a bleeding disorder in patients with a normal platelet count, but deficient clot retraction, and, until recently, these remained the only diagnostic criteria for the rare congenital disease which has come to bear his name. In the last few years, however, the platelets of such patients have been shown to fail to aggregate in response to any concentration of ADP or any other stimulus, and this absolute defect of platelet aggregation is now generally accepted as the chief characteristic that defines thrombasthenia (Hardisty *et al.*, 1964; Zucker *et al.*, 1966; Caen *et al.*, 1966). It is possible that the defect of clot retraction may result simply from the failure of platelets to aggregate during coagulation, as the retractile power of individual platelets is probably insufficient to produce normal retraction. It has been suggested that these platelets might have a deficiency of thrombosthenin, but this has not been substantiated (Weiss and Kochwa, 1968). On the other hand, several authors have demonstrated a deficiency of platelet fibrinogen in thrombasthenia (Nachman, 1966; Zucker *et al.*, 1966; Weiss and Kochwa, 1968), and it has been suggested that this might account for both the defective aggregation, for which fibrinogen is known to be essential, and the failure of clot retraction. Caen and his associates (1966), however, have found that some thrombasthenic platelets have relatively normal amounts of fibrinogen, yet have equally severe defects of aggregation and clot retraction.

The findings characteristic of thrombasthenia are summarised in Table 1. It seems likely that all the observed abnormalities are attributable to a single underlying biochemical defect, probably of the platelet surface membrane. This defect would result in a failure of the platelets to react with ADP, and all the other effects, with the possible exception of the fibrinogen deficiency,

would result from that. Demonstration of the nature of such a defect might well go a long way towards elucidation of the normal mechanism of platelet aggregation.

The chief clinical feature of thrombasthenia is a tendency to bruise on the

TABLE 1. Thrombasthenia

Abnormal	Normal
Bleeding time	Platelet count
Platelet aggregation by ADP, etc.	Platelet morphology, sphering by ADP
Platelet adhesion to glass	Platelet adhesion to connective tissue
Clot retraction	Thrombosthenin
Platelet fibrinogen (usually)	Platelet ATP and ADP (usually)
Release of platelet ADP by glass	Release of platelet ADP and 5 HT by connective tissue
Platelet factor 3 availability	Platelet factor XIII

slightest provocation and children are commonly covered in multiple superficial bruises: deep haematomata and haemarthroses, such as are seen in haemophilia, hardly ever occur. Superficial cuts and abrasions tend to bleed excessively and for a long time, epistaxes are common and menorrhagia is very often a serious problem in female patients. Heredity is of the autosomal recessive type. It might be thought that transfusion of normal platelets would be the logical method of treatment when serious haemorrhage occurs, or to cover tooth extractions and essential operations, but we have obtained rather disappointing results with this therapy (Hardisty *et al.*, 1964). Luckily, local measures are more effective than in the hereditary coagulation disorders.

SECONDARY PLATELET AGGREGATION AND ITS FAILURE

Platelets contain adenine nucleotides in high concentration, and ADP is normally released from platelets when they adhere to connective tissue (Hovig, 1963), and in response to the addition of extrinsic ADP (Macmillan, 1966), adrenaline (O'Brien, 1963), or certain other substances. This released ADP causes further platelet aggregation, as shown in Fig. 2; in this instance, the addition of ADP to platelet-rich plasma in final concentrations of up to 1·0 μM causes transitory and reversible aggregation, but a further slight increase in concentration above this results in a secondary wave of aggregation, due to the release of further ADP from the platelets themselves. In a number of unrelated clinical contexts, all of which may be associated with a bleeding tendency, this secondary aggregation fails to occur, owing apparently to a failure of the ADP release mechanism. The curves shown in Fig. 3 were obtained using the platelet-rich plasma of a boy aged eleven with a ventricular

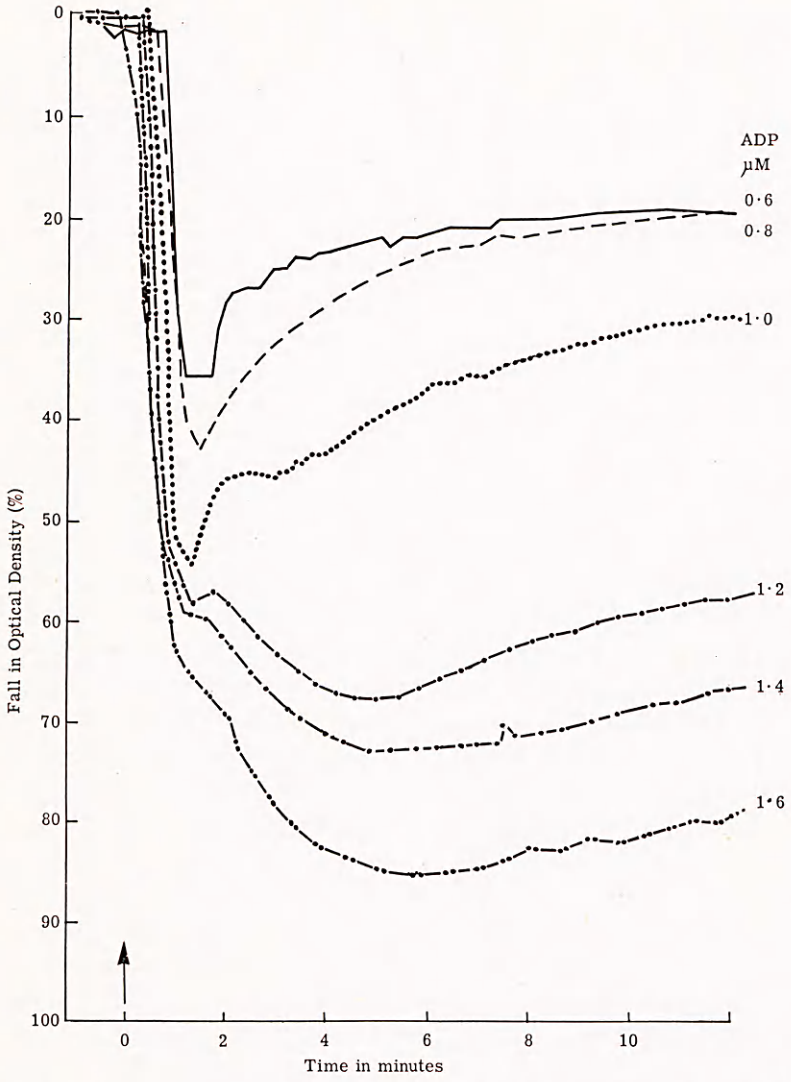


Fig. 2. The effect of various concentrations of ADP (added at zero time) on the optical density of normal platelet-rich plasma. A fall in optical density in this and the succeeding figs represents platelet aggregation, and a rise disaggregation.

septal defect and a constantly very prolonged bleeding time; he bruised easily and had had a number of episodes of bleeding following mild trauma. Despite the addition of high concentrations of ADP, which caused rapid and profound initial aggregation, no secondary aggregation occurred. Direct nucleotide estimations confirmed that the defect was one of ADP release from the

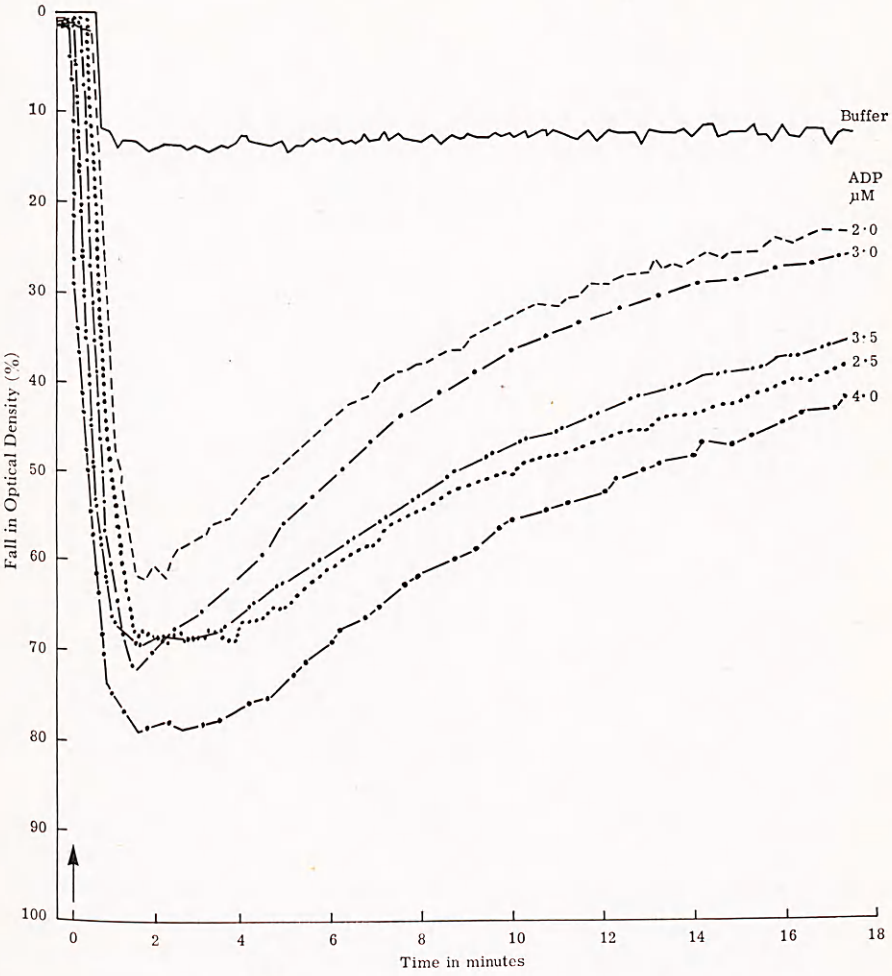


Fig. 3. The effect of various concentrations of ADP (added at zero time) on the optical density of platelet-rich plasma from a patient whose platelets exhibited no secondary aggregation.

platelets. This boy also has mild defects of platelet factor 3 availability and of platelet adhesiveness to glass, both of which are presumably secondary to this underlying defect of aggregation. He is one of about thirty patients with congenital heart disease in whom we have observed a long bleeding time, but in only one other such patient have we found a similar defect of platelet aggregation; no abnormality of platelet behaviour could be demonstrated in the remainder, in whom the long bleeding times remain unexplained.

This defect of ADP release can be demonstrated more strikingly by using adrenaline as the aggregating agent, since in this case the first phase is relatively small and the second phase usually much greater. In a normal subject (Fig. 4*a*), as little as 0·1 μM adrenaline causes massive secondary aggregation, but forty-eight hours after taking 500 mg of aspirin (Fig.4*b*), there is no secondary aggregation even with seven times this concentration. The effects of aspirin on platelets, which have been well described by Weiss and Aledort (1967), O'Brien (1968), and Zucker and Peterson (1968), may last for several days after a single dose of the drug, and it is important, whenever platelet function is being investigated, to enquire whether the patient has taken aspirin in any form during the preceding ten days. Aspirin ingestion commonly also prolongs the bleeding time, and the combination of its action on the gastric mucosa and on the platelets can sometimes lead to a dangerous clinical situation.

The various conditions in which this type of platelet defect has been observed are: (i) effects of aspirin and related drugs, (ii) uraemia, (iii) albinism, (iv) scurvy, (v) thrombocythaeria, and (vi) idiopathic congenital states. The haemorrhagic tendency which may occur in severe uraemia is complex in origin; there may be some degree of deficiency in plasma clotting factors, or of thrombocytopenia, but platelet functional defects are commonly involved and may be demonstrated by defective glass adhesiveness (Salzman and Neri, 1966) or abnormal platelet clotting function (Horowitz *et al.*, 1967). This failure of secondary aggregation has been observed in a number of uraemic patients (Hutton and O'Shea, 1968), and when it occurs, the aggregation defect is probably sufficient to account for the other observed platelet abnormalities. There is some evidence to suggest that the interference with platelet function in uraemia is due to the presence of an inhibitory substance in the plasma, perhaps some retained metabolite; Horowitz *et al.* (1967) have put forward the suggestion that this may be guanidinosuccinic acid, but other workers have failed to confirm this (Ten Cate, 1968). In any event, it appears to be true that correction of the uraemic defect by dialysis also corrects the platelet abnormality (Stewart and Castaldi, 1967).

Although the bleeding manifestations of scurvy have for a long time been thought to be due entirely to a vascular abnormality, defects of platelet function have recently been observed in man (Wilson *et al.*, 1967) and in scorbutic guinea pigs (Born and Wright, 1967). Defective platelet adhesiveness to glass has been the chief abnormality noted, but relatively little attention has been paid to platelet aggregation in this condition. In the only scorbutic patient I have had the opportunity of studying in this way, there was a marked defect of secondary aggregation. In thrombocythaemia, it is well known that

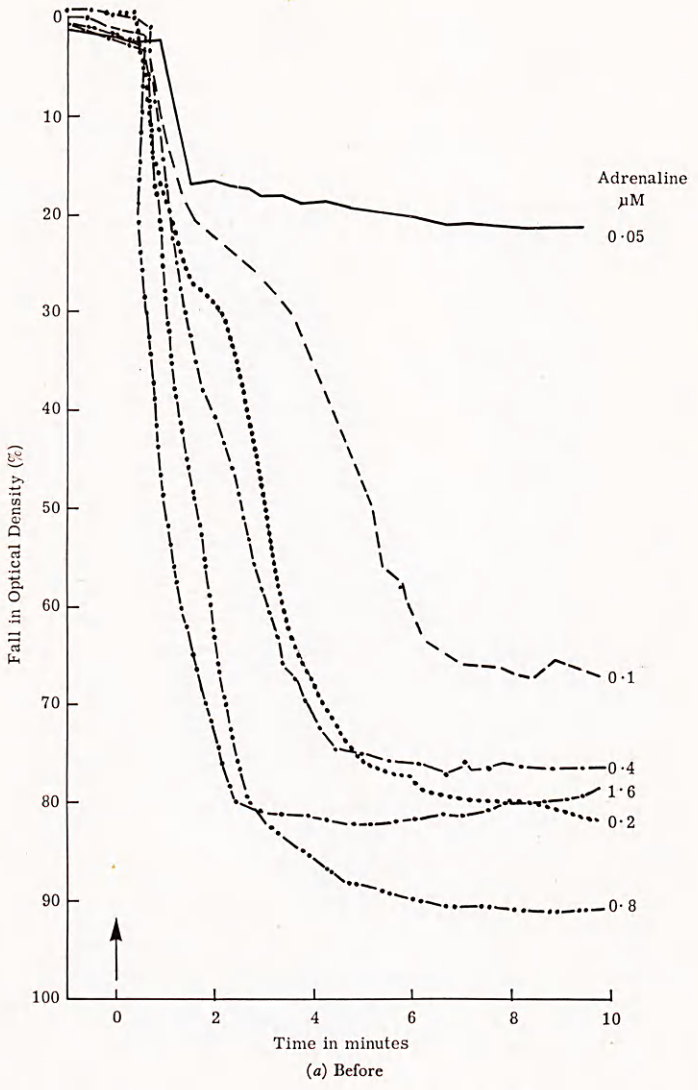
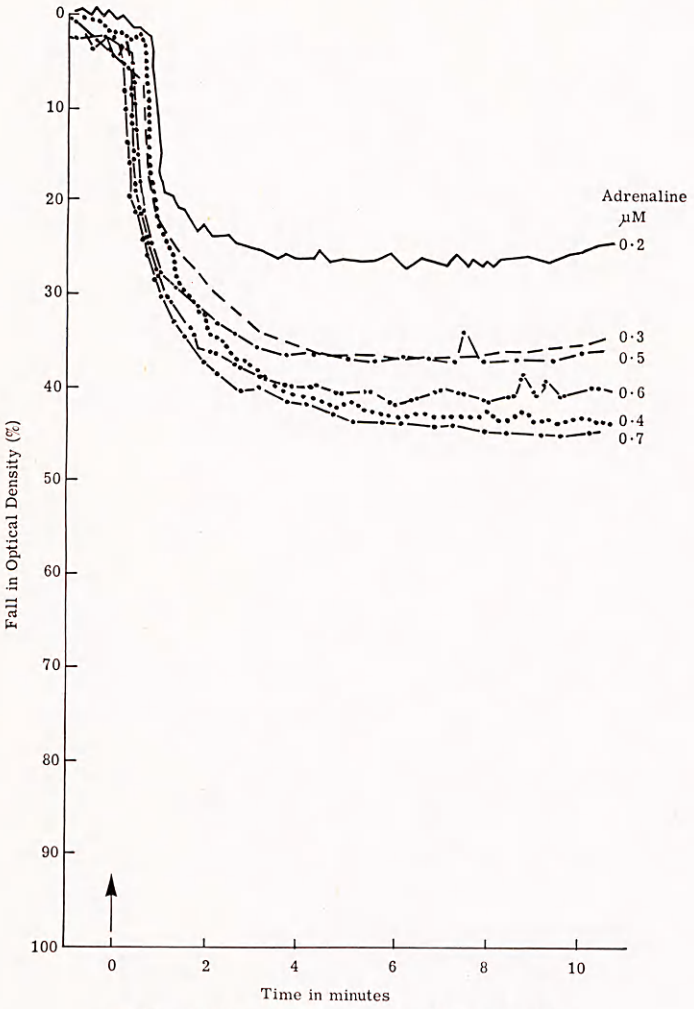


Fig. 4. The effect of various concentrations of adrenaline (added at zero time) on the optical density of platelet-rich plasma from a normal subject.

the very high platelet count is often associated with abnormal bleeding, which can usually be controlled by reducing the platelet count to within normal limits, and for a good many years there has been argument as to whether the abnormal clotting activity of the platelets in this condition was simply due to the excess of platelets present, or whether they were qualitatively abnormal.



(*b*) Forty-eight hours after the ingestion of 500 mg aspirin.

Fig. 4. *Cont'd.*

Recently, a defect of secondary aggregation in cases of thrombocythaemia has been observed by Spaet and Lejnieks (1968) and Inceman and Tangün (1968). Finally, this type of abnormality has been observed in a number of patients with apparently life-long mild bleeding disorders, including bruising, post-operative haemorrhage, and sometimes haematuria, for which no other explanation could be found, and which was apparently unrelated in any way to drug ingestion (Hardisty and Hutton, 1967; Weiss, 1967; Caen *et al.*,

1968). We have seen this in several sets of first-degree relatives of both sexes, and I therefore suggest that this failure of platelets to release ADP may sometimes occur as a genetically determined defect; substantiation of this will require many more quantitative studies of this phenomenon in the general population.

INTERFERENCE WITH PLATELET FUNCTION BY MACROMOLECULES

In a number of conditions circulating macromolecules may interfere with various platelet functions, probably by coating the surface of the platelets themselves, or of the connective tissue to which they normally adhere (Vigliano and Horowitz, 1967). These are dextran infusions, macroglobulinaemia, and myelomatosis. This is not the only mechanism leading to a bleeding tendency in these circumstances, as high molecular-weight dextran or abnormal globulins may also block the interaction of plasma clotting factors.

CONCLUSIONS

This paper represents an attempt to classify platelet abnormalities on a pathogenetic basis, and to indicate that seemingly complex disorders of platelet function can probably arise from a number of specific isolated defects. It is probably a gross over-simplification of the subject and, in the light of current research, will almost certainly require modification in the near future.

Acknowledgements

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Blackwell or Barry: A Question of Sex

Dr Shorthouse has pointed out that Elizabeth Blackwell may not have been the first woman doctor (*J. Roy. Coll. Phycns Lond.*, vol. 2, p. 105) a distinction which may belong to Dr James Barry who qualified at Edinburgh in 1812. This waspish little character served as an army doctor for over forty years, rising to the rank of Inspector General of Hospitals. A month after Barry died in 1865, the death certificate recording the sex as male, the *Manchester Guardian* published the startling statement that 'he' was a woman. The story was attributable to the charwoman who laid out the body and found it to be that of a 'normal female who had borne a child'; the doctor who certified death had identified but not examined the body. Immediately, various friends of Barry's remembered how feminine they had thought him to be but only one person recorded such an opinion during his lifetime, describing him as having the 'form, the manners, and the voice of a woman'.

Isobel Rae, Barry's biographer, who had access to all the official papers, was convinced that Barry was a woman, but he may well have been a hypogonadal male or a variety of intersex. Miss Blackwell has the distinction of qualifying as a known woman, whatever the actual sex of the unhappy and mysterious James Barry.