greatly extended. The principles are essentially so simple that there is no reason why other physicians should not achieve the same results even if they have not previously had the advantage of experience with more meticulous methods. It is important that tracer-dose investigations should always precede ^{131}I therapy, because they confirm the diagnosis, and ensure that the uptake of ^{131}I by the thyroid gland is high. Failing such tests it is inevitable that mistakes in diagnosis and management will occur.

The majority of patients who receive ^{131}I therapy would in the absence of the isotope be treated by partial thyroidectomy. This operation, including the pre-operative preparation and recovery, usually involves at least three weeks in hospital, with all the expense to the National Health Service that such an admission entails. Thereafter a shorter or longer period of convalescence is necessary before the resumption of ordinary activities. When patients are treated with ^{131}I in hospital they need not remain for longer than three or four days unless their general condition warrants it. Treatment of out-patients, on the other hand, involves no expense except that of the isotope itself. This is trivial in comparison with the costs of the hospital admission. Including transport charges, an average dose of 8 millicuries costs less than £2. The economies effected by isotope therapy are therefore considerable.

The elaborate methods previously used in an attempt to determine precisely the dose to be prescribed are expensive. The detailed measurements necessary, particularly when gland size is estimated by scanning techniques, involve physicists and technicians in an expenditure of time and effort probably unjustified by any advantage which accrues.

From the long-term point of view it is important that some centres with adequate facilities, and a sufficient staff of physicists and technicians, should continue to make quantitative observations upon the amount of radiation delivered in isotope therapy. Only then can further knowledge be obtained about the factors responsible for the variation in response. Effective and safe treatment, however, can be carried out at any centre with a minimum of equipment and technical personnel. The cases here reported have been treated by one physician with the assistance of a single technician. It therefore should not be thought that the application of radioactive iodine in the treatment of thyrotoxicosis is necessarily a complex procedure.

Summary

A series of 150 consecutive cases of thyrotoxicosis have been treated with radioactive iodine. A simple clinical dosage technique has been used, based primarily upon an approximate estimate, by inspection and palpation, of thyroid gland mass, although certain other clinical factors were also taken into consideration.

On review after a minimum period of one year, 87% of patients had become euthyroid, 11% were myxoedematous, and 2% had been inadequately treated. Fifty-nine per cent. of the patients were satisfactorily treated with a single dose of the isotope.

The results achieved did not differ appreciably from those previously reported in which a method had been employed for dose selection involving an elaborate and quantitative technique.

The prescription of an entirely accurate radiation dose is impracticable, chiefly because of the errors inherent in the techniques for the estimation of gland mass, and because of the biological variability in the radiosensitivity of different glands. Elaborate techniques are unnecessary for the safe and effective treatment of thyrotoxic patients.

I am grateful to Professor D. M. Dunlop for his interest in this work, and to Professor R. McWhirter for his co-operation. I

am indebted to the Secretary of State for Scotland, through the Advisory Committee on Medical Research, for a grant towards expenses.

REFERENCES

- Blomfield, G. W., Jones, J. C., Macgregor, A. G., Miller, H., and Wayne, E. J. (1951). *British Medical Journal*, 2, 373.
 ——— (1953). In *Radioisotope Techniques*, 1, 23. H.M.S.O., London.
 ——— and Weetch, R. S. (1955). *British Medical Journal*, 2, 1223.
 Chapman, E. M., and Maloof, F. (1955). *Medicine (Baltimore)*, 34, 261.
 Fraser, R., Abbott, J. D., and Stewart, F. S. (1954). *Brit. J. Radiol.*, 27, 23.
 ——— Hobson, Q. J. G., Arnott, D. G., and Emery, E. W. (1953). *Quart. J. Med.*, 22, 99.
 Freedberg, A. S., Kurland, G. S., Chamovitz, D. L., and Ureles, A. L. (1952). *J. clin. Endocr.*, 12, 86.
 Goodwin, J. F., Macgregor, A. G., Miller, H., and Wayne, E. J. (1951). *Quart. J. Med.*, 20, 353.
 Kelly, F. J. (1954). *J. clin. Endocr.*, 14, 326.
 Kelsey, M. P., Haines, S. F., and Keating, F. R. (1949). *Ibid.*, 9, 171.
 Miller, E. R. and Sheline, G. E. (1951). *Radiology*, 57, 720.
 Myant, N. B. (1953). *Brit. J. Radiol.*, 26, 139.
 ——— (1956). In *Therapeutic Use of Artificial Radioisotopes*, edited by P. F. Hahn, p. 148. John Wiley, New York.
 ——— and Pochin, E. E. (1955). In *British Practice in Radiotherapy*, edited by E. R. Carling, B. W. Windeyer, and D. W. Smithers, p. 382. Butterworth, London.
 Oddie, T. H. (1951). *Brit. J. Radiol.*, 24, 333.
 Soley, M. H., Miller, E. R., and Foreman, N. (1949). *J. clin. Endocr.*, 9, 29.
 Williams, R. H., Towery, B. T., Jaffe, H., Rogers, W. F., and Tagnon, R. (1949). *Amer. J. Med.*, 7, 702.

DANGEROUS BLEEDING ASSOCIATED WITH CARCINOMA OF PROSTATE

BY

H. T. SWAN, M.B., M.R.C.P., D.C.H.

Department of Haematology, the Royal Infirmary, Sheffield

K. F. WOOD, M.D., F.R.C.S.

AND

OWEN DANIEL, M.B., F.R.C.S.

University Department of Surgery and the Royal Infirmary, Sheffield

Excessive bleeding sometimes develops in patients with metastatic carcinoma of the prostate. The bleeding may occur either spontaneously or in association with surgical procedures, especially those directly involving the malignant prostate. Bleeding of varying degree may arise from any site, particularly the urinary tract and the nose, and in the skin as ecchymoses. If surgery is performed dangerous bleeding may take place into the traumatized area. Scott (1956) reports the loss of 1.5 litres of blood into the scrotum following orchidectomy in such a case. The haemorrhage may be severe enough to cause death if properly directed treatment is not given.

The relationship between bleeding and carcinoma of the prostate has not been fully explained. Recent studies of abnormal clotting have shown, however, that in certain cases of metastatic carcinoma of the prostate the phenomenon of fibrinolysis develops and that this is associated at times with a drop in the level of plasma fibrinogen (Tagnon *et al.*, 1953a, 1953b). Such fibrinolysis is thought to be due to proteolytic enzymes which dissolve away blood clot by digesting fibrin (fibrinolysis), in some cases also lysing fibrinogen (fibrinogenolysis) and other clotting factors such as prothrombin and factor V. Tagnon *et al.* (1953b) have demonstrated an abnormal fibrinolysin in the blood of 12% of their patients with metastatic carcinoma of the prostate, and

they have shown that this proteolytic enzyme is produced by the carcinomatous tissue itself.

In the following two cases of metastatic carcinoma of the prostate profuse bleeding occurred, sufficient to endanger life.

Case 1

First Admission

A 60-year-old man was admitted to hospital on December 27, 1953, with acute urinary retention and one month's history of urinary frequency, urgency, dribbling, and haematuria. Examination revealed a bladder distended to the umbilicus and enlarged nodes in the left groin. The left lobe of the prostate was enlarged, hard, and irregular, and a clinical diagnosis of carcinoma of the prostate was made. The patient was catheterized and 2 pints (1,140 ml.) of clear urine was obtained. A few hours after this procedure he started to bleed from the nose, rectum, and bladder. The bleeding was of moderate degree and did not require blood transfusion, but it lasted for four days.

Investigations.—Hb, 86% (12.7 g. per 100 ml.); W.B.C., 8,800 per c.mm.; platelets (Lempert), 77,000 per c.mm.; blood urea, 46 mg. per 100 ml.; serum acid phosphatase, 35 Gutman units; serum alkaline phosphatase, 29 K.A. units; plasma albumin, 3.1 g. per 100 ml., globulin, 2.9 g. per 100 ml., fibrinogen "practically zero." The patient's recalcified plasma formed a tiny pellicle of fibrin which disappeared during overnight incubation at 37° C. This test was not carried out aseptically, but even without proof of sterility the evidence was very suggestive of abnormal fibrinolysis.

On December 31, while he was still bleeding, he began treatment with stilboestrol, 10 mg. t.d.s., and this dose was increased to 15 mg. t.d.s. five days later. This therapy coincided with, or was responsible for, the cessation of all bleeding. Moreover, his platelet count, which had remained between 50,000 and 70,000 per c.mm. while he was bleeding, rose steadily to 230,000 per c.mm. over the succeeding five days and remained at that level. Subsequently his urological symptoms improved so much that there was no further need to consider transurethral prostatectomy.

Second Admission

Seven months later, while still receiving stilboestrol, 15 mg. t.d.s., the patient was admitted again with acute urinary retention. On this occasion he looked pale, and his chest, abdomen, and right wrist were covered with petechiae. Urine obtained by catheter was clear.

Investigations.—Hb, 60% (8.9 g. per 100 ml.); W.B.C., 7,900 per c.mm.; platelets, 86,000 per c.mm.; blood urea, 52 mg. per 100 ml.; serum acid phosphatase, 68 Gutman units; serum alkaline phosphatase, 33 K.A. units. Radiographs of the pelvis revealed secondary deposits in the bones, but the chest films showed no abnormality.

On the day after his readmission the patient suddenly collapsed, complaining of pain in the right shoulder and scapular region. He began to bleed round his indwelling catheter, and blood began to ooze from a venepuncture wound of the previous day. His nose started to bleed, and further showers of petechiae appeared on his arms and trunk. He became very shocked, and his clinical condition was not improved by the transfusion of three bottles of stored and one of fresh blood. Eight hours after the onset of bleeding he died from shock due to exsanguination from massive bowel haemorrhage.

A post-mortem examination confirmed the presence of a poorly differentiated adenocarcinoma of the prostate penetrating the capsule. There were metastases in the lungs, ribs, vertebrae, and pelvis. Petechiae were seen on the serosal surface of the gall-bladder, on the submucosal surface of the renal pelvis, and in the mucosa of the bladder as well as the skin, and there were haemorrhages into the wall of the pharynx, liver, and bowel. No local lesion was found to account for the large amount of blood in the alimentary tract.

Comment

At this time (1954) the condition of hypofibrinogenaemia associated with fibrinolysis in cases of carcinoma of the prostate was not recognized by us. It is possible, however, that fibrinogen depletion and fibrinolysis caused the haemorrhagic tendency which ultimately proved fatal to the patient even though this diagnosis is substantiated by relatively few haematological investigations.

Case 2

On February 13, 1956, a man aged 71 presented with haematuria which had developed over the previous week. He gave a history of six months of unremitting backache and of scalding and frequency of micturition. The liver was palpable 1½ in. (3.8 cm.) below the costal margin. He had a large fixed prostate gland which was thought to be probably malignant. By the time of his admission to hospital on March 6 the haematuria had stopped spontaneously.

Investigations

Hb, 97% (14.4 g. per 100 ml.); total serum acid phosphatase, 220 Gutman units; serum alkaline phosphatase, 30 K.A. units; serum bilirubin 1.1 mg. per 100 ml.; thymol turbidity, 1 unit; total serum proteins, 6.5 g. per 100 ml. Radiographs of the pelvis did not show any evidence of bony metastases, but an intravenous pyelogram revealed a moderately severe left hydronephrosis and a lobulated filling defect on the bladder base.

As an initial therapeutic procedure bilateral orchidectomy was performed, and a perineal biopsy of the prostate was also taken. This ultimately confirmed the presence of an anaplastic carcinoma of the prostate. After the biopsy had been taken all the traumatized tissue began to bleed freely, and the patient developed peripheral circulatory failure. Post-operatively the perineal wound continued to bleed, and his condition deteriorated. No improvement was observed as a result of the emergency transfusion of three bottles of stored blood over four hours; indeed, he began to develop spontaneous subcutaneous bruising around the shoulder-girdle and arms. At this stage it was reported that the original specimen of venous blood removed for the emergency cross-matching had still failed to clot. The syndrome was fully investigated by the following laboratory tests.

1. *Failure of Blood to Clot.*—The specimen of blood removed immediately after the operation had not been in contact with an anticoagulant, and yet it appeared to remain completely fluid apart from a very minute clot which comprised little more than a few fibrin strands lying loose within the specimen. This tiny clot did not increase in size thereafter. Indeed, it became less visible during the succeeding five hours.

This single observation, which was made in the blood bank, was enough to suggest the diagnosis of fibrinogen depletion in such a patient. The following emergency test confirmed the presumptive diagnosis.

2. *Thrombin Plus Whole Blood.*—Since thrombin acts directly on fibrinogen and by-passes all the earlier clotting factors, it is, in the absence of inhibitors, a specific indicator of the presence of fibrinogen. 0.2 ml. of commercial thrombin (Maw, 50 N.I.H. units per ml. of normal saline) was added to the same volume of oxalated whole blood (Scott, 1955). When assessed some 60 seconds later a very poor clot had formed. It was small in size, occupied a mere fraction of the total volume, and was ineffective in enmeshing red cells. By contrast, normal blood produced a large firm clot occupying the whole volume and enmeshing almost all the red cells.

When the original specimen which had been sent for cross-matching without an anticoagulant was then used in place of the oxalated specimen, and thrombin was added, no clot at all was formed. This showed the complete absence of fibrinogen. One presumes that such fibrinogen as had been present originally had all gone to form the few strands of fibrin described above.

This simple thrombin test demonstrated hypofibrinogenaemia, and this was enough to be the basis of specific emergency therapy. It did not supply information about the presence or absence of fibrinolysis such as affects formed clots. Perhaps the simplest demonstration of abnormal fibrinolysis occurs when sterile clotted blood liquefies spontaneously within 24 hours, but in this case lack of fibrinogen precluded the test in that form.

Treatment of the Bleeding.—Dried fibrinogen kept in readiness by the Sheffield Regional Blood Transfusion Service for such an occasion as this was used. This had been fractionated from pooled human plasma. About 4 g. was dissolved in 200 ml. of pyrogen-free distilled water and given to the patient intravenously over 20 minutes. At that time he was dangerously shocked, with blood running freely from the wound, and had a weak pulse of 130 a minute. All serious bleeding ceased within half an hour, and within one hour of the fibrinogen transfusion all oozing had stopped, with dramatic clinical improvement. No further fibrinogen therapy ever became necessary. The wound subsequently healed well.

Subsequent Investigations

The time interval between operation and removal of blood for the test described is given in parentheses.

1. **Platelet count** (Lempert) (12 hours), 150,000 per c.mm.

2. **Whole-blood Clotting-time** (Lee and White).—(12 hours):—A clot formed at about 20 minutes. This was still not a whole-volume clot, and it shrank considerably, until two hours later it was scarcely apparent amongst the red cells.

3. **Thrombin Plus Whole Blood.**—(12 hours):—Repetition of this test for the presence of fibrinogen (see above) showed a moderate-sized but nevertheless incomplete clot when examined at 60 seconds. The clot was much larger than it had been the previous night. (60 hours):—Identical with the control.

4. **Serial Plasma Dilution with Added Thrombin** (0, 12, 60 hours).—This is a simple emergency method for fibrinogen assay as advocated by Wolf (1954). The principle of the test is evident from the Table.

Serial Plasma Dilutions

Dilution in Saline:	1:10	1:20	1:40	1:80	1:160	1:320
Patient's plasma:						
Before fibrinogen therapy (0 hrs.)	+	+	—	—	—	—
After fibrinogen therapy (12 hrs.)	+	+	+	+	—	—
After fibrinogen therapy (60 hrs.)	++	++	+	+	+	+
Normal plasma as control (0, 12, and 60 hrs.)	++	++	+	+	+	+

++ = large clot, + = small or very small clot. Formed by the addition of thrombin to all tubes.

It will be appreciated that the clot formed by the addition of thrombin to a dilution of normal plasma of 1:320 is very minute and, to be seen, requires close scrutiny in a good light. From the Table the fibrinogen level in the patient's plasma can be calculated on the basis of a dilution factor of $\times 2$ between each tube. Thus, if one assumes the control fibrinogen level to be 200–400 mg. per 100 ml., then the pre-fibrinogen therapy specimen of the patient would be 12.5–25 mg. per 100 ml. and the post-therapy specimen would be 50–80 mg. per 100 ml. An estimate of fibrinogen by the Kjeldahl method later showed this second specimen to have in fact a fibrinogen content of 80 mg. per 100 ml. It thus confirmed the approximate range as given by this emergency method. The latter, however, took only 20–30 minutes to perform and read, as compared with hours by the Kjeldahl method.

5. **Estimation of Plasma Fibrinogen by Kjeldahl Method.**—At 12 hours, 80 mg. per 100 ml.; at 60 hours, 390 mg. per 100 ml.

6. **Quantitative Test for Fibrinolysis** (Biggs and Macfarlane, 1953a).—(12 hours):—Serial estimations showed no evidence of commencing fibrinolysis up to four hours. The blood was not kept chilled before the test. (60 hours):—A minimal increase of lysis was found after 19 hours when compared with the control, but the difference was not regarded as significant.

7. **Examination for Lysis of Normal Clot by Patient's Plasma.**—(12 hours):—No lysis was visible to the naked eye following the immersion of a washed fibrin clot (formed by

the addition of thrombin to normal plasma) into undiluted patient's plasma, followed by incubation at 37° C. for 24 hours.

8. **Quick's One-stage "Prothrombin" Time.**—(12 hours):—Control 15", patient 25", prothrombin index 60%, prothrombin arbitrary efficiency 18%. A prolongation of the "prothrombin" time may be expected in the presence of a low fibrinogen level, but this does not exclude the possibility of other defects (see below).

9. **Two-stage Prothrombin Time** (Biggs and Douglas, 1953a).—(36 hours):—True prothrombin was 77% of normal. This level is within our normal range allowed by the method.

10. **Qualitative Test for Deficiency of Factor V or of Factor VII** (Biggs and Macfarlane, 1953b).—(12 hours):—No deficiency of factor V or of factor VII.

11. **Thromboplastin Generation Test** (Biggs and Douglas, 1953b).—(36 hours):—No abnormality demonstrated by this test in the patient's plasma, serum, or platelets.

Discussion

In Case 2 it was demonstrated that a dangerous fall occurred in the plasma fibrinogen level during a minor operative procedure on a malignant prostate gland followed by bilateral orchidectomy. Furthermore, the plasma fibrinogen rose to an adequate haemostatic level after a single intravenous infusion of 4 g. of human fibrinogen. The plasma fibrinogen was only 80 mg. per 100 ml., however, some hours after all bleeding had ceased, and this level is lower than that which is commonly believed to be haemostatic. The plasma fibrinogen then continued to increase, apparently spontaneously, until it reached normality on the third day. This recovery may have been assisted by the orchidectomy. The bleeding in Case 1 appeared to develop in the absence of any surgical trauma to the prostate gland, unless catheterization can be described as such.

Fibrinolytic activity was probably present in Case 1, as shown by the complete lysis of the clot left overnight. In Case 2 it is unfortunate that the first specimen examined specifically for fibrinolysis was taken from the patient 12 hours after bleeding had stopped as the result of treatment. By then there was no satisfactory evidence of fibrinolysis by the method used. Possibly a modification of the cold method advocated by Fearnley and Lackner (1955) might have given more information. Hypofibrinogenaemia therefore remains the unequivocal feature of these two patients. Fibrinolysis remains a probability in Case 1 but an unproved factor in Case 2.

Tagnon *et al.* (1953b) have shown in cases of metastatic carcinoma of the prostate with fibrinolysis that, while the platelet count is often depressed, it may remain normal despite the fibrinolysis. In Case 1 the platelet count was abnormally low at the time of the bleeding and recovered subsequently during oestrogen therapy. The exact significance of this is not clear.

An unusual feature present in Case 2 was the apparently isolated fall in plasma fibrinogen. There was no obvious fibrinolytic activity to which proteolytic digestion of fibrinogen could be attributed. Nor do we know of any evidence to suggest that the mechanism is the same as that found in premature separation of the placenta (Stevenson *et al.*, 1953) and perhaps in certain lung operations (Chalnot *et al.*, 1952), where it seems that intravascular clotting may take place with consequent depletion of fibrinogen, as a result of the release into the circulation of excessive thromboplastin from the organ involved. The fibrinolytic factor, when it is present, is more easily understood, since tissue juices from a prostatic carcinoma have been shown *in vitro* to cause clot lysis and also to digest fibrinogen, factor V (ac-globulin), and probably prothrombin (Tagnon *et al.*, 1953a, 1953b).

Therapy is directed to controlling fibrinolysis and correcting hypofibrinogenaemia. In practice these two may perforce have to be treated together. Oestrogen therapy has an important place in controlling the lytic factor when present

(Tagnon *et al.*, 1953a) and this is substantiated by Case 1. The position in Case 2 is less certain. Also it seems that removal of the source of testosterone by orchidectomy has an experimental justification over and above the mere need to make the female hormone preponderant over the male hormone, since it was shown by Tagnon *et al.* (1953a) that the addition of testosterone to a maintenance dose of oestrogen made the latter ineffective in their patient. Corticotrophin has also been claimed to diminish the lysis, thereby helping to control the bleeding manifestations in these patients, and Stefanini *et al.* (1953) claimed that response to this treatment was more rapid than with oestrogens. Therapy of the hypofibrinogenaemia is extremely important, and must be as specific as possible. It will be appreciated that whole blood is relatively useless for this purpose, since it supplies so little fibrinogen in each bottle. Fractionated fibrinogen or triple-strength reconstituted dried plasma is the only type of emergency therapy. If whole blood must be given to replace blood loss, fibrinogen infusion should be given first, or at least simultaneously. About 4 g. of fibrinogen should be given intravenously in the first place, and subsequent additions to this should depend on the patient's recovery and on laboratory findings. As Case 2 has shown, one injection alone may be sufficient.

In conclusion, therefore, we suggest that the association of abnormal bleeding and carcinoma of the prostate is not infrequent, and, when suspected, should be confirmed by the more simple tests described in Case 2. The control of hypofibrinogenaemic bleeding depends on its recognition and the immediate availability of human fibrinogen.

Summary

Two cases are described in which the patients suffered from metastatic carcinoma of the prostate and developed a generalized haemorrhagic state. During these episodes the blood of both patients was incoagulable owing to a deficiency of plasma fibrinogen. In the first patient, who died as a result of the bleeding, this loss was probably associated with a fibrinolytic mechanism. The second patient recovered dramatically on receiving 4 g. of human fibrinogen intravenously.

Simple methods are described for the emergency laboratory diagnosis of hypofibrinogenaemia, using commercial bovine thrombin.

Whole blood is almost valueless as a specific therapy for hypofibrinogenaemia, and should be used only for its bulk, its cellular constituents, and its other clotting factors. Hypofibrinogenaemia should be treated by fractionated fibrinogen intravenously or by triple-strength reconstituted dried plasma.

The treatment of fibrinolysis is discussed.

We thank Professor R. P. Jepson for permission to describe these patients, who were under his care; Dr. H. Lempert, of the Royal Infirmary, Manchester, for allowing us to use the results of his haematological investigations in the first patient; and Dr. E. K. Blackburn for his help. We are indebted to Miss Pauline Hall for technical assistance, and to the Endowment Fund of the Board of Governors of the United Sheffield Hospitals for a grant towards the cost of laboratory expenses.

REFERENCES

- Biggs, R., and Douglas, A. S. (1953a). *J. clin. Path.*, **6**, 15.
 — (1953b). *Ibid.*, **6**, 23.
 — and Macfarlane, R. G. (1953a). *Human Blood Coagulation and its Disorders*, p. 358. Blackwell, Oxford.
 — (1953b). *Ibid.*, p. 352.
 Chalmot, P., Michon, P., and Mme Lochard (1952). *Rev. Hémat.*, **7**, 25.
 Fearnley, G. R., and Lackner, R. (1955). *Brit. J. Haemat.*, **1**, 189.
 Scott, E. van Zile (1956). *J. Urol.*, **75**, 116.
 Scott, J. S. (1955). *British Medical Journal*, **2**, 290.
 Stefanini, M., Campbell, E. W., Plitman, G. I., and Salomon, L. (1953). *Bull. New Engl. med. Cent.*, **15**, 23.
 Stevenson, C. S., Braden, R. G., Schneider, C. L., Johnson, J. F., and Seegers, W. H. (1953). *Amer. J. Obstet. Gynec.*, **65**, 88.
 Tagnon, H. J., Schulman, P., Whitmore, W. F., and Leone, L. A. (1953a). *Amer. J. Med.*, **15**, 875.
 — Whitmore, W. F., Schulman, P., and Kravitz, S. C. (1953b). *Cancer (N.Y.)*, **6**, 63.
 Wolf, P. (1954). *British Medical Journal*, **2**, 1226.

PROPHYLACTIC VALUE OF TETANUS ANTITOXIN IN MICE PREVIOUSLY INJECTED WITH HORSE SERUM

BY

LEON S. KIND, Ph.D.

From the Department of Microbiology, Medical College of South Carolina, Charleston, S.C.*

Littlewood, Mant, and Payling Wright (1954) reported a case in which a patient died of tetanus despite the fact that he was given the generally accepted prophylactic dose of tetanus antitoxin (1,500 units) shortly after injury. The patient was shown to be allergic to tetanus antitoxin (via a positive skin test), and his case history revealed that he had been given a prophylactic dose of tetanus antitoxin after an injury six months previously. Littlewood *et al.* suggested that the therapeutic ineffectiveness of the tetanus antitoxin was due to the state of hypersensitivity to the antitoxin which resulted in its accelerated removal from the circulation. They cited data from investigations of Glenney and Hopkins (1924), Talmage *et al.* (1951), and others which clearly indicate that previous experience with an antigen results in an accelerated removal of that antigen from the circulation upon its subsequent injection. The following experiment tests the hypothesis of Littlewood *et al.* by noting the duration of the prophylactic effectiveness of tetanus antitoxin in normal mice and in mice previously injected with horse serum.

Methods

Female 22–30 g. Carworth Farms CF strain of mice were divided into three groups. Group I was injected intraperitoneally with 0.1 ml. of *Haemophilus pertussis* vaccine† and 0.5 ml. of undiluted horse serum. Groups II and III received only 0.1 ml. of pertussis vaccine. Normal horse serum rather than horse-serum antitoxin was used to sensitize mice to a subsequent injection of antitoxin, since preliminary experiments indicated the superiority of normal horse serum as a sensitizing antigen. This result was not surprising in view of the fact that the antitoxin employed (Lederle) was of the type that is treated with proteolytic enzymes to modify its allergenic properties. Pertussis vaccine was administered along with the horse serum since it has been shown by Malkiel and Hargis (1952), Kind (1953), and Munoz *et al.* (1954) that this vaccine increases the sensitivity of mice to anaphylactic shock. Eleven days later the mice in groups I and II were inoculated intraperitoneally with 0.2 ml. (300 units) of tetanus antitoxin.

Of 62 mice sensitized with horse serum and injected with the antitoxin, 2 died and 4 others showed symptoms of severe anaphylaxis but recovered. On the third, seventh, fifteenth, nineteenth, and twenty-fourth day after receiving antitoxin, 12 mice from group I and 12 mice from group II were challenged intraperitoneally with approximately 1 M.L.D. of tetanus toxin. On each of these days 12 mice from group III (previously injected with only pertussis vaccine) were similarly challenged with tetanus toxin. The tetanus toxin employed in this experiment was obtained from Lederle Laboratories (lot No. 1872M). The fluid toxin had been stored at refrigerator temperatures for two years, but possessed considerable potency; 0.1 ml. of a 1:200 dilution injected intraperitoneally was approximately 1 M.L.D. Toxin dilution was made in nutrient broth, and deaths were noted for four days after injection.

*This investigation was supported by research grant (E372C2) from the National Microbiological Institute, National Institutes of Health, Public Health Service, Department of Health, Education, and Welfare.

†48.8 billion organisms per ml.