(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.

Results

The data in the Table and the Chart indicate that up to the fifteenth day after the administration of tetanus antitoxin all mice possessed sufficient antitoxin to neutralize the

Effect of Previous Administration of Horse Serum on Duration of Protective Ability of Tetanus Antitoxin

Group	On Day 0	On Day 11	No. of Mice Surviving Challenge (Survivors/Total) with Approximately 1 M.L.D. of Tetanus Toxin				
			Days After Administration of Antitoxin*				
			3	7	15	19	24
I	0.1 ml. pertussis vaccine + 0.05 ml. horse serum	0.2 ml. tetanus antitoxin (300 units)†	12/12	12/12	9/11	4/13‡	2/12
II	0.1 ml. pertussis vaccine	"	12/12	12/12	12/12	11/12‡	2/12
III	"	—	2/12	1/12	2/11	0/12	0/12

* Note that the mice in group III received no antitoxin; they served as controls for the potency of the toxin.

† Two mice died of anaphylactic shock; 4 showed symptoms of severe anaphylaxis and recovered.

‡ The difference in the percentage of survivors in these two groups is statistically significant ($P < 0.01$).

Duration of prophylactic effectiveness of tetanus antitoxin in mice inoculated with horse serum and in mice not so inoculated.

challenge dose of toxin. Of the mice challenged on the fifteenth day, two deaths occurred among the animals of group I which had been inoculated with horse serum 11 days prior to antitoxin. No deaths occurred among the animals which had no previous experience with horse serum (group II). Of the mice challenged on the nineteenth day after the administration of antitoxin, only 4 of 13 (30%) of the animals inoculated with horse serum survived the challenge dose of tetanus toxin, as compared with 11 of 12 (91%) of the mice which had not previously received horse serum. The difference between the percentage of survivors in the two groups is a significant one ($P < 0.01$). In a second experiment conducted in a similar manner, comparable results were obtained. Of 23 mice inoculated with horse serum and challenged with tetanus toxin on the seventeenth day after the administration of tetanus antitoxin, only 7 survived. In contrast, of 24 mice with no previous experience with horse serum, 22 survived challenge doses of toxin administered on the seventeenth day after injections of antitoxin. The difference between the percentage of survivors in the two groups is a significant one ($P < 0.01$).

Discussion

The data presented here support the contention of Littlewood *et al.* that previous experience with horse serum may

result in failure of antitoxin to protect. However, we agree with Beeson (1955-6) that an alternative explanation of the fatal case of tetanus cited by the aforementioned authors may be that the conventional prophylactic dose of tetanus antitoxin (1,500 units) was insufficient to neutralize the toxin present. Beeson cites other cases of prophylactic failure of 1,500 units of tetanus antitoxin and suggests that 10,000 units should be used for adequate passive immunization.

An increase in the prophylactic doses of tetanus antitoxin from 1,500 to 10,000 units would thus seem to be advantageous for two reasons. Ten thousand units would offer better protection in cases in which unusually large amounts of toxin were being produced. Ten thousand units of tetanus antitoxin might also be more effective in cases in which the antitoxin was being rapidly eliminated because of the patient's previous experience with horse serum.

REFERENCES

- Beeson, P. B. (1955-6). *Year Book of Medicine*. The Year Book Publishers, Chicago.
 Glenny, A. T., and Hopkins, B. E. (1924). *J. Hyg. (Camb.)*, 22, 208.
 Kind, L. S. (1953). *J. Immunol.*, 70, 411.
 Littlewood, A. H. M., Mant, A. K., and Wright, G. Payling (1954). *British Medical Journal*, 2, 444.
 Malkiel, S., and Hargis, B. J. (1952). *Proc. Soc. exp. Biol. (N.Y.)*, 80, 122.
 Munoz, J., Schuchardt, L. F., and Verwey, W. F. (1954). *Fed. Proc.*, 13, 507.
 Talmage, D. W., Dixon, F. J., Bukantz, S. C., and Dammin, G. J. (1951). *J. Immunol.*, 67, 243.

A SIMPLE QUICK TEST FOR GLUCOSE IN URINE

REPORT ON USE OF CLINISTIX

BY

GEORGE LUNTZ, M.R.C.P.

Consultant Chest Physician, Birmingham Chest Clinic;
 Consultant Physician with Charge of Tuberculosis
 Diabetic Service, Birmingham Regional
 Hospital Board

Two extremely simple enzyme tests have been developed which are highly specific for glucose in urine. These are "tes-tape" and "clinistix," but the present paper is concerned only with the latter. The method consists in dipping a specially treated thick paper stick approximately the size of a book-match, the last half-inch of which is impregnated with reagents containing an enzyme system, into a specimen of urine. The stick turns blue in the presence of glucose, and the blue colour should have developed in one minute. If the stick remains colourless there is no significant glucose in the specimen.

Application of the Test

This paper reports on a field trial in which clinistix was used for detecting glucose in urine and the effectiveness of the test compared with "clintest" reagent tablets as standard. The trial was limited to patients over the age of 11 years attending the Birmingham Chest Clinic for the first time. Nursing staff were responsible for obtaining a specimen of urine from all new patients in the specified group. This was normally done in the interval between the taking of the chest radiograph and the clinical examination, so that the tests were completed before the patient was seen by the physician. Nurses performed the actual tests after being trained in the technique, and recorded the results. The technique is simple, no special apparatus or boiling is required, and specificity is beyond doubt. Hunt, Gray, and Thorogood (1956) have already stressed that the test is essentially an ultra-micro-method in the strictest sense and requires all the standards of chemical cleanliness such methods demand.

In the small number of patients whose specimens gave a positive result, blood-sugar estimations were carried