Glycosuria was found in only one child, a known diabetic. It is interesting to note that 10 instances of childhood urinary infection have now been found in this practice and only one diabetic, and that urinary tract infection with structural or functional abnormalities of the renal tract is apparently at least five times as common as diabetes in childhood.

We wish to thank all those nurses, receptionists, secretaries, and technicians who made this study possible. Special thanks are due to Mrs. D. Spencer, S.R.N., Mrs. L. Mitchell, S.R.N., and Mrs. P. A. L. Mond. Dr E. Grundy, Medical Officer of Health, London Borough of Brent, kindly arranged for the use of clinic facilities.

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Requests for reprints should be sent to Dr. R. N. Grünberg.

REFERENCES

- Fry, J., Dillane, J. B., Joiner, C. L., and Williams, J. D. (1962). *Lancet*, 1, 1318.
 Gallagher, D. J. A., Montgomerie, J. Z., and North, J. D. K. (1965). *British Medical Journal*, 1, 622.
 Kunin, C. M., Deutscher, R., and Paquin, A., jun. (1964). *Medicine*, 43, 91.
 Leigh, D. A., and Williams, J. D. (1964). *Journal of Clinical Pathology*, 17, 498.
 Loudon, I. S. L., and Greenhalgh, G. P. (1962). *Lancet*, 2, 1246.
 Meadow, S. R., White, R. H. R., and Johnston, N. M. (1969). *British Medical Journal*, 3, 81.
 Mond, N. C., Percival, A., Williams, J. D., and Brumfitt, W. (1965). *Lancet*, 1, 514.
 Randolph, M. F., and Greenfield, M. (1964). *Journal of Pediatrics*, 65, 57.
 Savage, D. C. L., Wilson, M. I., Ross, E. M., and Fee, W. M. (1969). *British Medical Journal*, 3, 75.
 Smellie, J. M., Hodson, C. J., Edwards, D., and Normand, I. C. S. (1964). *British Medical Journal*, 2, 1222.
 Smellie, J. M., and Normand, I. C. S. (1966). *Proceedings of the Royal Society of Medicine*, 59, 415.

Pleural Effusion and Fibrosis during Treatment with Methysergide

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Summary: Two patients being treated for migraine with methysergide developed extensive pleural fibrosis, and in addition one of them had bilateral pleural effusions. After treatment was stopped these complications, which are thought to have been due to the drug, cleared in the next few months.

Introduction

Methysergide (Deseril) is effective in the prevention of severe migraine but has many side effects, among which are peculiar fibroses affecting the retroperitoneal tissue, heart valves, coronary arteries, and the myocardium (Ureles and Rob, 1963; Graham, 1964; Sweetnam, 1964; *Lancet*, 1965; Graham *et al.*, 1966). Graham (1967) first suspected a relation between otherwise unexplained pleuropulmonary disease, and similar cases have been reported by Bays (1968) and Lindeneg and Kok-Jensen (1968).

We record below two cases of pleuropulmonary complications which we believe to be due to methysergide and which to the best of our knowledge are the first to be reported from this country.

Case 1

A 50-year-old storeman, who had suffered from migraine for many years and been treated with methysergide 1 mg. t.d.s. for six months, was admitted to hospital because of increasing dyspnoea and abdominal pain. Dullness to percussion and decreased air entry were found at both bases, and chest x-ray examination confirmed the presence of bilateral pleural effusions with evidence of fibrosis.

No asbestos bodies, malignant cells, or tubercle bacilli were found in the sputum. Blood-stained pleural fluid was aspirated from the right pleural space and clear fluid from the left side. Neither specimen contained tubercle bacilli or malignant cells. Pleural biopsy showed dense hyaline fibrous tissue but no evidence of malignancy.

Investigations showed haemoglobin 12.5 g./100 ml., E.S.R. 65 mm. in one hour (Westergren), and total serum proteins 6.6 g./100 ml. (albumin 2.9 g., globulin 3.7 g.). Protein strip showed decreased albumin and alpha-2 globulins. Rose-Waaler and lupus erythematosus latex tests were negative and no L.E. cells were seen. Serum calcium and phosphate were normal.

Intravenous pyelogram gave no evidence of retroperitoneal fibrosis and barium meal examination was normal. Liver and muscle biopsy showed no abnormality.

Methysergide was stopped and since then there has been a gradual lessening of the dyspnoea with pronounced improvement in the chest x-ray appearances and a fall in the E.S.R. to 28 mm. in one hour.

Case 2

A 37-year-old woman schoolteacher was referred for chest x-ray examination because of pain in her left lower chest and back for four months, associated with dyspnoea on effort. In 1956 sarcoidosis was proved on gland and liver biopsy, but there was never any evidence of pulmonary or pleural involvement. She had been treated with steroids for two years, there being no subsequent signs or symptoms of sarcoidosis. For six years she had been treated with methysergide (3-6 mg./day) for severe attacks of migraine.

On examination there was a loud crackling friction rub at the left base and her left lower back was tender. No other abnormalities were found. Haemoglobin (14.5 g./100 ml.) and W.B.C. were normal, and E.S.R. was 33 mm. in one hour (Westergren). Serum aspartate aminotransferase 66 i.u./ml., but serum proteins, electrolytes, and alkaline phosphatase were normal. Latex test was negative and no L.E. cells were seen. Mantoux test was negative at 1:100.

Chest x-ray film showed extensive pleural fibrosis at the left base with high tenting of the left diaphragm. There was no definite evidence of pleural effusion. Intravenous pyelogram was normal.

Methysergide was stopped and her respiratory symptoms rapidly improved. Seven months later she felt well, the friction rub had disappeared, and her E.S.R. was 10 mm. in one hour and her serum aspartate aminotransferase 10 i.u./ml. She had no further attacks of migraine. Within two months of stopping methysergide her chest x-ray picture showed clearing of the lesions at the left base with the exception of obliteration of the left costophrenic angle and some fibrosis in the left interlobar fissure.

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Discussion

A causal relationship between methysergide treatment and otherwise unexplained retroperitoneal fibrosis—a rare condition—has now been widely accepted. In conditions as common as pleural effusions and fibrosis the diagnosis of drug-induced disease is more difficult to prove and rests mostly on circumstantial evidence. In both of our cases pleural effusions and fibrosis developed while the patient was receiving methysergide, and rapid improvement followed when the drug was stopped.

The most striking feature in these cases was the degree of radiological clearing of pleural fibrosis after cessation of methysergide therapy, particularly in Case 2. This is an uncommon occurrence in pleuritis of other origins but in accordance with the regression observed in retroperitoneal fibrosis due to methysergide.

The number of people treated for migraine is considerable

and presumably many of them receive methysergide. In cases of otherwise unexplained "pleurodynias," pleural effusions, and fibrosis the possibility of drug induction by methysergide should be considered.

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REFERENCES

- Bays, R. P. (1968). *Journal of the Louisiana State Medical Society*, **120**, 426.
 Graham, J. R. (1964). *New England Journal of Medicine*, **270**, 67.
 Graham, J. R. (1967). *American Journal of the Medical Sciences*, **254**, 1.
 Graham, J. R., Suby, H. I., LeCompte, P. R., and Sadowsky, N. L. (1966). *New England Journal of Medicine*, **274**, 359.
Lancet, 1965, **1**, 1262.
 Lindene, O., and Kok-Jensen, A. (1968). *Nordisk Medicin*, **79**, 681.
 Sweetnam, M. (1964). *Practitioner*, **193**, 792.
 Ureles, A. L., and Rob, C. (1963). *Journal of the American Medical Association*, **183**, 1041.

Preliminary Communications

Isotope Bioassay for "Thrombopoietin"

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Summary: An assay system has been developed in mice for the humoral agent "thrombopoietin." This is based on utilization in newly-formed platelets of the radio-amino-acid ^{75}Se selenomethionine 24 hours after intravenous injection. The possibility that the results could be due to the effects of species antibody, to foreign protein, or to an effect primarily on selenomethionine metabolism, has been explored, but the findings are consistent with the presence of a physiological humoral factor. Thrombopoietin has been shown to be present in the plasma of patients suffering from severe thrombocytopenia of varied aetiology.

INTRODUCTION

Evidence suggesting the existence of a new humoral agent, "thrombopoietin," has been reviewed in earlier publications (de Gabriele and Penington, 1967a, 1967b). This agent, which is analogous to the physiological regulator of red cell production, "erythropoietin," appears to subserve a physiological role in the regulation of platelet production.

Hitherto attempts to demonstrate the presence of the hormone in plasma have rested on finding a raised platelet count in recipient animals, the increases being relatively small, of the order of 20 to 25% over the resting platelet count (Kelemen *et al.*, 1958; Linman *et al.*, 1959; Rák *et al.*, 1959; Odell *et al.*, 1961; Spector, 1961; Schulman *et al.*, 1965; McClure and Choi, 1968). Errors inherent in all visual methods of counting platelets are considerable even under the most carefully controlled conditions (Hellem, 1960), and in many of the studies referred to above the technique used for counting platelets is not quoted; the possibility of observer bias in visual counting methods is always present unless specific precautions are taken to avoid this.

The results reported have been very far from consistent, with claims of raised thrombopoietin in polycythaemia with thrombocytosis (Linman *et al.*, 1959) and in both polycythaemia vera and essential thrombocythaemia (Kelemen *et al.*, 1961); these are reminiscent of the early conflicting reports of the role of erythropoietin in similar conditions using assays

dependent on visual counting of red cells and reticulocytes. Many of these findings were subsequently disproved once an objective and quantitative assay system for erythropoietin was available, using the isotope ^{59}Fe . Abildgaard *et al.* (1967), working in Schulman's laboratory, noted difficulty in reproducing their earlier demonstration of thrombopoietin based on platelet counting in rats, and there is clearly a need for a reproducible and quantitative method of assay for thrombopoietin. Until such a method is available little progress can be made in studying the chemical and physiological nature of the hormone, and confusion will remain regarding its role in the genesis of human disease.

The radio-amino-acid, ^{75}Se selenomethionine, has been shown to label in vivo the formed elements of the blood, including platelets (Cohen *et al.*, 1965; Penner, 1966), and Evatt and Levin (1968) have reported, in abstract form, that plasma from thrombocytopenic rabbits increased the percentage utilization of the label in the circulating platelets of recipient animals. This technique has now been applied to establish a bioassay for thrombopoietin in human plasma.

SUBJECTS AND METHODS

The techniques of platelet counting, sampling, labelling with ^{75}Se selenomethionine, and calculation of utilization of the isotope were as previously described (Penington, 1969). Male C57 mice weighing 20-26 g. were injected with 0.002 ml. of an antiserum to mouse platelets (prepared in rabbits as described previously) one week before beginning the assay. The animals were injected subcutaneously with varying doses of plasma from thrombocytopenic donors. The maximum dose for a single injection was 0.5 ml., given subcutaneously under light anaesthesia. The timing of injections of plasma and ^{75}Se selenomethionine varied in the different studies as indicated. In a small group of animals the assay was carried out without previous injection of antiplatelet serum.

The routine assay procedure consisted of twice-daily injections of 0.1 or 0.5 ml. of plasma into groups of five animals for two days, the animals having been prepared with antiplatelet serum a week before the first injection. On the third day 2 μCi of ^{75}Se selenomethionine was injected intravenously, and determination of the utilization of the isotope in platelets was performed 24 hours later by the method previously described (Penington, 1969).