parathyroid-hormone-stimulated bone resorption by a direct effect on the bone of intact and thyroparathyroidectomized rats. The experiments of Tanzer *et al.* (1970), using radio-phosphorus labels in rats, also support the concept of a direct action of glucagon on bone. Glucagon may, therefore, have a similar action to mithramycin in Paget's disease—namely, inhibiting resorption while still allowing the anabolic effect of parathyroid hormone on bone to continue (Condon *et al.*, 1971).

The third possible mode of action of glucagon on bone is more speculative. A large number of enzymes have been found to increase after glucagon therapy, and one of these, glucose-6-phosphatase (Langdon and Weakley, 1955), is an acid phosphatase that has also been shown to be a pyrophosphatase (Nordlie and Arion, 1964; Stetten and Taft, 1964; Nordlie, 1965). Bone contains a number of pyrophosphatases, some of which may be involved in bone resorption (Russell and Fleisch, 1970). Fleisch and his co-workers have proposed that pyrophosphate, an inhibitor of calcification, binds to apatite and inhibits its formation (Fleisch *et al.*, 1966a) as well as its dissolution (Fleisch *et al.*, 1966b). Pyrophosphatases catalyse the hydrolysis of pyrophosphate and by destroying the pyrophosphate locally allows calcium to be either deposited or resorbed. The rate of bone formation and resorption are probably finely balanced by means of an unidentified mechanism (Harris and Heaney, 1969), which may involve pyrophosphate and a number of pyrophosphatases. Stimulation of certain pyrophosphatases by glucagon may in some way affect the local mechanisms controlling the rate of bone formation and resorption and account for the clinical and biochemical changes induced by glucagon.

If this last explanation is correct glucagon may prove to be useful in investigating the role of different pyrophosphatases in normal and pathological bone metabolism, and this may eventually lead to a clearer understanding of the aetiology of a number of bone disorders.

I am grateful to Dr. J. Jenkins, Dr. J. Nixon, Mr. Bendall, and Dr. J. Batten for their permission to report Cases 1, 2, 3, and 4.

I am also indebted to Dr. G. V. Foster and Dr. M. B. Clark for the calcitonin assays and to Professor J. A. Owen and Dr. P. Maitland and staff of the department of chemical pathology for their help. I would also like to thank Eli Lilly Ltd. for their generous assistance.

References

- Avioli, L. V., Birge, S. J., Kanagawa, H., and Shieber, W. (1968). *Journal Clinical Investigation*, **47**, 3a.
- Bijvoet, O. L. M., van der Sluys Veer, J., and Jansen, A. P. (1968). *Lancet*, **1**, 876.
- Care, A. D., Bates, R. F. L., and Gitelman, H. J. (1970a). *Journal of Endocrinology*, **48**, 1.
- Care, A. D., *et al.* (1970b). *Journal of Endocrinology*, **48**, 667.
- Clark, M. B., Boyd, G. W., Byfield, P. G. H., and Foster, G. V. (1969). *Lancet*, **2**, 74.
- Condon, J. R., *et al.* (1971). *British Medical Journal*, **1**, 421.
- Fleisch, H., Maerki, J., and Russell, R. G. G. (1966a). *Proceedings of the Society for Experimental Biology and Medicine*, **122**, 317.
- Fleisch, H., Russell, R. G. G., and Straumann, F. (1966b). *Nature*, **212**, 901.
- Foster, G. V. (1968). *New England Journal of Medicine*, **279**, 349.
- Friedman, J., and Raisz, L. G. (1965). *Science*, **150**, 1465.
- Gehrke, C. W., Affprung, H. E., and Lee, Y. C. (1954). *Analytical Chemistry*, **26**, 1944.
- Grant, R. A. (1964). *Journal of Clinical Pathology*, **7**, 685.
- Harris, W. H., and Heaney, R. P. (1969). *New England Journal of Medicine*, **280**, 193, 253, 303.
- Kind, P. R. N., and King, E. J. (1954). *Journal of Clinical Pathology*, **7**, 322.
- Kraml, M. (1966). *Clinica Chimica Acta*, **13**, 442.
- Langdon, R. G., and Weakley, D. R. (1955). *Journal of Biological Chemistry*, **214**, 167.
- MacIntyre, I. (1960). *Advances in Clinical Chemistry*, **4**, 1.
- Nordlie, R. C. (1965). *Journal of Biological Chemistry*, **240**, 2155.
- Nordlie, R. C., and Arion, W. J. (1964). *Journal of Biological Chemistry*, **239**, 1680.
- Paloyan, E., Paloyan, D., and Harper, P. V. (1967). *Metabolism*, **16**, 35.
- Pennock, C. A., Moore, G. R., and Hoyle, M. D. (1970). *Journal of Medical Laboratory Technology*, **27**, 302.
- Reynolds, J. J. (1968). *Proceedings of the Royal Society*, **B170**, 61.
- Russell, R. G. G., and Fleisch, H. (1970). *Clinical Orthopaedics and Related Research*, **69**, 101.
- Stern, P. H., and Bell, N. H. (1970). *Endocrinology*, **87**, 111.
- Stetten, M. R., and Taft, H. L. (1964). *Journal of Biological Chemistry*, **239**, 4041.
- Tanzer, F. S., Kennedy, J. W., and Talmage, R. V. (1970). *Proceedings of the Society for Experimental Biology and Medicine*, **133**, 500.
- Woodhouse, N. J. Y., *et al.* (1971). *Lancet*, **1**, 1139.

PRELIMINARY COMMUNICATIONS

Unusual Particles in Splenic Disorders

A. E. STUART, A. I. S. MACPHERSON,
J. RICHMOND

British Medical Journal, 1971, **4**, 721-722

Summary

Virus-like particles have been seen on electronmicroscopic examination of four spleens surgically removed for haematological disorders—three cases of idiopathic thrombocyto-

penic purpura and one case of haemolytic anaemia. The particles were approximately spherical and 50-60 nanometres in diameter.

During the course of electron microscopic examination of surgically resected human spleens, virus-like particles were seen in three cases of idiopathic thrombocytopenic purpura and in one case of haemolytic anaemia of obscure origin. Our material was derived from nine patients—single cases of congenital spherocytosis, haemolytic anaemia, myelofibrosis, and disseminated lupus erythematosus associated with thrombocytopenia, and three cases of idiopathic thrombocytopenic purpura. Two normal spleens were also examined. The clinical histories of four patients are summarized.

Case Reports

Case 1.—Man aged 22. Sudden onset of spontaneous bleeding, purpura, and ecchymosis. No history of drugs or recent illness. Spleen not palpable. Haemoglobin 15 g/100 ml, platelets under 10,000/mm³, W.B.C. 6,400/mm³, differential count normal. Marrow showed numerous megakaryocytes. Idiopathic thrombocytopenic

University of Edinburgh Medical School, Edinburgh

A. E. STUART, F.R.C.P.ED., PH.D., Reader in Pathology
J. RICHMOND, M.D., F.R.C.P.ED., Reader in Medicine

Royal Infirmary, Edinburgh EH3 9YW

A. I. S. MACPHERSON, CH.M., F.R.C.S.ED., F.R.S.ED., Consultant Surgeon

purpura diagnosed and prednisolone 60 mg/day given for three weeks. This reduced the bleeding tendency, but increased the platelets to only 55,000. At operation a spleen which was normal in appearances but slightly enlarged (330 g) was removed. Subsequent progress excellent. Six months later symptom-free, platelets 310,000.

Large numbers of virus-like particles from Case 1. Note the dark central core surrounded by an electron lucent shell. ($\times 30,000$.)

Case 2.—Married woman aged 26. Ten weeks previously had treated a sore throat with a mixture of aspirin, phenacetin, codeine, and caffeine. Two weeks later sudden onset of ecchymoses and menstrual period prolonged. Spleen not palpable. Capillary fragility increased. Haemoglobin 12 g/100 ml, platelets 16,000/mm³, W.B.C. 7,400/mm³, differential count normal. Marrow hypercellular with many megakaryocytes. Withdrawal of drugs and treatment with prednisolone 60 mg/day led to rapid remission with platelets rising to 206,000, but on slow withdrawal of prednisolone platelets fell to 48,000. Diagnosis was thought to be idiopathic rather than secondary thrombocytopenic purpura. At operation a small (90 g) normal-looking spleen was removed. Symptom-free since operation. One year later platelets 250,000.

Case 3.—Man aged 29. Sudden onset of spontaneous bruising and purpura. No history of drugs or previous illness. Platelets 31,000/mm³, bleeding time prolonged, spleen not palpable. Marrow showed many megakaryocytes. No response to three-week course of prednisolone 45 mg/day. Splenectomy followed by complete remission. Six months after operation platelets 210,000.

Case 4.—Woman aged 50 suffering from haemolytic anaemia was a neurasthenic. Treated for years with chlorpromazine, Drinamyl, and imipramine. One year before operation her haemoglobin was 9 g/100 ml, reticulocytes 2-7%, platelets 260,000/mm³, W.B.C. 4,000/mm³, normal differential count, and bilirubin 2-3 mg/100 ml. Marrow was hyperactive with no abnormal constituents. Osmotic fragility increased. Coombs test, lupus erythematosus cells, antinuclear factor, and Schumm's test

all negative. Serum folate, serum vitamin B₁₂, and haptoglobin normal. Withdrawal of all drugs led to no improvement. Prednisolone 4 mg/day for three months had no effect on blood count or symptoms. Anti-I test done at this time was normal, but anti-i was positive at 1,000 times dilution. At operation a moderately enlarged (350 g) spleen was removed and liver biopsy specimen taken. This was normal except for pronounced haemosiderosis. After splenectomy the platelets and leucocytes increased, but no improvement in red cell count. The haemoglobin continued at about 8 g/100 ml, reticulocytes 2-6%. After transfusion of 4 pints (2.3 litres) of packed cells haemoglobin rose to 13.6 g, but within seven weeks had fallen to 9 g. The cause of the haemolytic anaemia was obscure but was thought to be an inborn error of erythropoiesis of the type described by Crookston *et al.* (1969).

Comment

The particles measured 50-60 nanometres in diameter. They were approximately spherical and had an electron dense core surrounded by an outer shell. They were abundant and readily found, and were situated in the cytoplasm of reticular cells and were often seen as buds projecting from the cytoplasmic membrane. Sometimes small groups were found within the cytoplasm remote from the cell membrane (see Fig.). They were usually associated with reticular cells, less often with the cytoplasm of immunoblasts in germinal centres. Some were seen on the long processes of reticular cells where they apparently bridge across into the cytoplasm of neighbouring immunoblasts. These curious structures seem too small to be confused with lysosomes and their morphology does not fit with secretory granules.

Prince and Adams (1966) showed that platelet lysosomes might simulate virus particles. The spleen is rich in platelets and this source of error ought seriously to be considered. However, their illustrations lend no support to the view that our particles are derived from platelets. Likewise they are much smaller than the serotonin granules of White (1971). Their distribution around the periphery of cells suggests they might be pinocytotic vesicles. Against this is their structure and also their presence in small groups remote from the cell membrane. Clinically the particles were found in all three cases of idiopathic thrombocytopenic purpura but not in the case of thrombocytopenia secondary to disseminated lupus erythematosus. They were absent in the two normal spleens and in the cases of spherocytosis and myelofibrosis. We freely acknowledge that the sample is small, but nevertheless our preliminary experience suggests that these particles are either found easily or not at all. We emphasize the necessity for extreme caution in ascribing a viral nature to these particles until they have been propagated and isolated in culture. Furthermore, their relevance to the pathogenesis of splenic disease is unknown. It may be that the human spleen harbours commensal and latent virus more often than we suspect.

References

- Crookston, J. H., *et al.* (1969). *British Journal of Haematology*, 17, 11.
- Prince, A. M., and Adams, W. R. (1966). *Journal of the National Cancer Institute*, 37, 153.
- White, J. G. (1971). *American Journal of Pathology*, 63, 403.