Reverse transformation seemed to affect only tumour cells, since no effect was observed in the normal skin, mucousae, or other epithelial tissues. The selective character of this action explained the absence of toxicity of thioproline treatment.

The lack of activity of thioproline in experimental transplanted rodent tumours was not considered a setback. Serial transplantation might have caused important modifications of cell membranes which, while apparently not affecting tumour growth and response to cytotoxic drugs, might have impaired the response to the normal regulatory mechanisms of cell function that depend on membrane receptors.

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5. Konzentrat SRK [human], Zentral Lab. Blutspendedienst SRK, Switzerland. The freeze-dried material in each ampoule was dissolved in 8 ml of twice-distilled water, and this yielded a preparation containing 7.5 mg/ml of fibrinogen and 434.5 units of factor VIII per ml (one unit of factor VIII is defined as the amount contained in 1 ml of fresh normal plasma).

6. The freeze-dried material in each ampoule was dissolved in 8 ml of twice-distilled water, and this yielded a preparation containing 7.5 mg/ml of fibrinogen and 434.5 units of factor VIII per ml (one unit of factor VIII is defined as the amount contained in 1 ml of fresh normal plasma).

7. The supernatant of the saline wash was combined with the infranatant fluid obtained after the second entrapment and a few glass beads were added and the flask was shaken centrifugation at 50 000 g for 10 min. The liposomes were pelleted and diluted to a volume of 50 ml with isotonic saline before oral administration. A more detailed description of the method has appeared elsewhere.

8. We used factor VIII prepared by cryoprecipitation (AHF Konzentrat SRK [human], Zentral Lab. Blutspendedienst SRK, Switzerland). The freeze-dried material in each ampoule was dissolved in 8 ml of twice-distilled water, and this yielded a preparation containing 7.5 mg/ml of fibrinogen and 434.5 units of factor VIII per ml (one unit of factor VIII is defined as the amount contained in 1 ml of fresh normal plasma).

9. The supernatant of the saline wash was combined with the infranatant fluid obtained after the second entrapment and factor VIII and fibrinogen were determined. It seemed that this fluid contained 19-22% of the original factor VIII and 76-79% of the fibrinogen. This shows that the liposome preparation is indeed preferentially enriched with factor VIII, probably because of interaction between factor VIII and the lipid bilayer shells.

10. Plasma factor-VIII activity rose on all three occasions when a liposome preparation of factor VIII was given before breakfast to a patient with severe haemophilia (mean factor VIII level when not on treatment less than 0.5% of normal, no circulating antibodies). The results in one of the three experiments are shown in the accompanying figure. Plasma concentrations after the same amount of factor VIII had been administered intravenously are also shown. The patient had haematuria before ingestion of the liposome preparation; this disappeared on the day of the experiment and returned on the third day after the experiment. Plasma factor-VIII activity did not rise when either liposomes or factor VIII were administered separately.

Some of the factor VIII administered in the liposomes appeared in the plasma as factor-VIII activity. It took

ORAL TREATMENT OF HæMOPHILIA A BY GASTROINTESTINAL ABSORPTION OF FACTOR VIII ENTRAPPED IN LIPOSOMES

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Summary Factor-VIII-loaded liposomes were given orally to a patient with severe haemophilia A. Plasma concentrations of factor VIII rose to therapeutically effective levels, that persisted for 50 hours.

INTRODUCTION

Hæmophilia A is caused by a sex-linked congenital lack of functional coagulation factor VIII. Coagulation factor VIII is a plasma protein, as yet not well characterised but associated with a protein complex with a molecular weight of 2 000 000. In current treatment and prophylaxis of haemophilia A, partly purified preparations of this protein are administered intravenously. We describe a preparation consisting of liposomes loaded with factor VIII that, when administered orally to a haemophilic patient, produced a rise in plasma factor VIII procoagulant activity.

Liposomes are artificial structures that consist of multiple concentric bilayers of phospholipids. Proteins may be entrapped in the interstices between the bilayers. When liposomes are prepared in the presence of an aqueous solution of an enzyme, 5-15% of the enzyme or enzyme-protein may become enclosed in these structures. Liposome-entrapped proteins entered intact cells and insulin-loaded liposomes administered orally caused a drop in blood glucose in diabetic rats. Because factor VIII has been reported to interact hydrophobically with phospholipids when involved in blood coagulation, we thought that it might be possible preferentially to absorb factor VIII on phospholipids and in this way obtain liposomes with a high factor-VIII content in which factor VIII was relatively stable.

Despite problems associated with oral administration of matter contained in liposomes, we thought that because of the specific binding of factor VIII to lipids, administration of factor-VIII-loaded liposomes to a haemophilic patient might raise the plasma factor VIII content.

METHODS

We prepared factor-VIII-loaded liposomes by shaking a factor VIII solution with glass beads in a flask, the wall of which had been coated with phospholipids. A 200 ml round-bottomed flask was coated with 250 mg of egg lecithin containing 5% (w/w) of phosphoric acid, by addition of lecithin in ethanol and evaporation of the solution to dryness in a rotary evaporator under reduced pressure. Then 5 ml of the factor VIII solution and a few glass beads were added and the flask was shaken gently until all lipid was removed from the flask wall.

The suspension obtained was centrifuged for 30 min at 27 000 g at 10°C which caused the liposomes to float. The aqueous phase was taken out with a hypodermic syringe and used to make a second liposome preparation as described above. Both liposome preparations were pooled and washed once with isotonic saline followed by centrifugation at 50 000 g for 10 min. The liposomes were pelleted and diluted to a volume of 50 ml with isotonic saline before oral administration. A more detailed description of the method has appeared elsewhere.

We used factor VIII prepared by cryoprecipitation (AHF Konzentrat SRK [human], Zentral Lab. Blutspendedienst SRK, Switzerland). The freeze-dried material in each ampoule was dissolved in 8 ml of twice-distilled water, and this yielded a preparation containing 7.5 mg/ml of fibrinogen and 434.5 units of factor VIII per ml (one unit of factor VIII is defined as the amount contained in 1 ml of fresh normal plasma).

The supernatant of the saline wash was combined with the infranatant fluid obtained after the second entrapment and factor VIII and fibrinogen were determined. It seemed that this fluid contained 19-22% of the original factor VIII and 76-79% of the fibrinogen. This shows that the liposome preparation is indeed preferentially enriched with factor VIII, probably because of interaction between factor VIII and the lipid bilayer shells.

RESULTS

Plasma factor-VIII activity rose on all three occasions when a liposome preparation of factor VIII was given before breakfast to a patient with severe haemophilia (mean factor VIII level when not on treatment less than 0.5% of normal, no circulating antibodies). The results in one of the three experiments are shown in the accompanying figure. Plasma concentrations after the same amount of factor VIII had been administered intravenously are also shown. The patient had haematuria before ingestion of the liposome preparation; this disappeared on the day of the experiment and returned on the third day after the experiment. Plasma factor-VIII activity did not rise when either liposomes or factor VIII were administered separately.

Some of the factor VIII administered in the liposomes appeared in the plasma as factor-VIII activity. It took
Oral administration of factor VIII may give better results than intravenous injection. The apparently lower effect on clotting activity immediately after ingestion is compensated by the maintenance of therapeutic concentrations on the second day after administration. Since concentrations of between 5 and 10% of normal are usually sufficient for prophylaxis and treatment of minor bleeds and concentrations of 25–35% are necessary for the treatment of severe bleeds and concentrations of 50% have been required for the treatment of haemophilia A. For the same reason we can report here on experiments in one patient only.

DISCUSSION

Oral administration of factor VIII may give better results than intravenous injection. The apparently reduced effect on clotting activity immediately after ingestion is compensated by the maintenance of therapeutic concentrations on the second day after administration. Since concentrations of between 5 and 10% of normal are usually sufficient for prophylaxis and treatment of minor bleeds and concentrations of 25–35% are sufficient for major bleeds and surgical cover, in practice the efficacy of a dose of factor VIII given orally may be as high as that of the same dose given intravenously.

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advances in human genetics


this series is now well established as providing authoritative reviews of growing points in human genetics, and the current volume is no exception. d. g. hardin and a. m. r. taylor's chapter on chromosomes and neoplasia includes not only well-documented descriptions of cytogenetic abnormalities associated with various neoplasms, but also speculation on the possible relevance and significance of these findings in regard to human cancer. this is an eleven-page chapter that is both informative and precise. the critical examination, by j. m. opitz and colleagues, of the vexed problems of terminology and nomenclature of birth defects is a tour de force in this notoriously confusing field. to some, arguments over definitions may seem pedantic, but precision is essential if the etiology of these malformations is ever to be understood. the discovery in recent years of the diagnostic value of alpha-fetoprotein levels in serum for hepatocarcinoma, and in amniotic fluid for neural-tube defects, reflects perhaps as much shortcomings of reproductive standards, not "a virologist's virus", it has, nevertheless, a double interest, on the one hand as the causal organism of glandular fever, and on the other as the first virus known to cause human cancer, and it is this which has led to the intense work volume is no exception. d. g. hardin and a. m. r. taylor's chapter on alpha-fetoprotein levels in serum for hepatocarcinoma, and in amniotic fluid for neural-tube defects, has been a source of much debate in the field of human virology. it should be cherished by virologists, studied by oncologists, and certainly not ignored by cell biologists.

electron microscopy of the kidney in renal disease and hypertension


this readable textbook is characterised by an abundance of electronmicrographs. the first chapter, on microscopy for the physician, is both factual and helpful and, as the author notes, is derived from leeson and leeson's histology. the chapter on the clinical assessment of the anatomy and pathological activity of glomerulonephritis seems irrelevant, since it is intended to be a guide to the biochemical investigation of glomerulonephritis. that on the pathogenesis and classification of glomerulonephritis is clear and well written. others are rather superficial—for example, the one on the "pathology of the kidney in pyelonephritis, pyelonephritis" (sic), which covers diverse subjects. the electronmicrographs and occasional other illustrations vary considerably in quality, their defects reflecting perhaps as much shortcomings of reproduction as deficiencies in the original plates. membranous glomerulonephritis, a condition which is superbly illustrated in most general textbooks, is very poorly illustrated here. in short, this book differs from the classical texts of hamburger, heptinstall, or zollinger, in that it is neither balanced nor comprehensive. it has the merit of being more lavishly illustrated by electronmicrographs than any of these texts, although many of the electronmicrographs scarcely merit inclusion.