luxation is more common than we think is tenable, I cannot say. The onus lies with those who treat their patients without fusion to show that their method is as safe as surgery.

DR. A. B. MYLES (*Chertsey*) Before advising a patient with a symptomless subluxation that he requires surgical treatment, we should be sure that those who have symptomatic subluxation and cord compression benefit by this treatment. You have not told us your results. In my experience, patients who have refused operation have done rather better than those who have had the operation, and they have not, as expected, continued to deteriorate. One or two have died subsequently with other diseases and, although considerable subluxation has been found at autopsy, there is such laxity, that there is no longer any compression on the cord, and it is not clear that they are at risk.

MR. SWEETNAM This is marvellous. Here we have someone who is presumably not going to submit any more of his patients to surgery. Now it is beholden upon him to provide us with a prospective study in 10 years' time, and then we shall have the answer which we are all seeking.

Reference

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Effect of a Fibrinolytic Agent (Arvin) on Wound Healing and Collagen Formation. By P. J. L. HOLT, V. HOLLOWAY, N. RAGHUPATI, and J. S. CALNAN (*Departments of Medicine and Experimental Surgery, Royal Postgraduate Medical School*)

A purified fraction of the venom of the Malayan pit viper (trade name Arvin) is capable of producing complete defibrinogenation. This is not associated with the bleeding which occurs if heparin is used.

This drug was used to assess the effect of defibrinogenation on wound healing and connective tissue formation. Standard wounds were produced on the backs of rabbits and inert implants placed in the subcutaneous tissue of the flanks. Almost complete defibrinogenation was produced, plasma fibrinogen at all times being less than 50 mg./100 ml. Similar undefibrinogenated rabbits were used as controls, with a further group of animals in which the plasma fibrinogen had been artificially raised.

The wound strength and histology, the weight and histology of new tissue formation around the implants, and the histology of untouched skin were compared in the three sets of animals.

Defibrinogenation produced impaired wound healing and defective connective tissue formation. Alterations in collagen and other skin structures in specimens of untouched skin were also found.

To elucidate the nature of these changes, in particular the part played by Arvin directly and indirectly in defibrinogenation, were investigated *in vitro* and by the use of further animal models.

Discussion

DR. D. L. GARDNER (*Kennedy Institute*) These subjects

are undoubtedly controversial, and I should like to ask Dr. Holt how he preserved the tissues, the microscopic preparations of which he illustrated and from the appearance of which he deduced that ground substance formation was diminished under Arvin treatment.

DR. HOLT They were all preserved in formol saline.

DR. D. L. GARDNER (*Kennedy Institute*) It is necessary to be extremely careful in drawing conclusions on the quantity of ground substance present in a tissue from the study of microscopic preparations processed through paraffin. Engfeldt and Hjertquist (1967, 1968) have demonstrated by the use of $^{35}\text{SO}_4$ that enormous losses of matrix sulphated glycosaminoglycan quickly succeed fixation in formaldehyde or glutaraldehyde followed by postossification and dehydration. In such preparations, up to 70 per cent. of $^{35}\text{SO}_4$ may be lost in the 4 hours preceding embedding.

DR. HOLT I was aware of this, but it came out after we had gone through the work, and I have said that we are less certain of the ground substance until we have some chemical measurements.

DR. H. MUIR (*London*) The histological changes produced by Arvin on the intact skin in adult animals occurred within 7 days as judged from your last slides. The effect would, therefore, appear to be on pre-formed collagen, whereas the effect in wound healing is on newly-formed collagen. Can you explain this?

DR. HOLT We tried a system of putting in an implant at minus 4, minus 3, minus 2, and minus 1 week into the control animal, and into some that were to be defibrinogenated. From this we could find what an implant which has been in for 4 weeks should weigh and so on. What we hope to do, and this is very difficult, is (having got our standards) to defibrinogenate one week after the last implant is inserted and see what happens to it. Suppose, for example, that an implant has been in for 1 week; will it gain weight normally, will it stay the same weight, or will the weight fall? As I have said, we had difficulty with our collagen estimations and extractions, but, judging the weight alone, we think it is following a straight line; in other words, no new collagen is being laid down. This work is very difficult because there are such wide variations.

DR. H. MUIR (*London*) Has Arvin any effect on collagen *in vitro* in the test tube in the absence of fibrinogen? Does Arvin have any direct effect on soluble collagen?

DR. HOLT No. What we have done is add Arvin by itself, Arvin with serum (plasma is of course instantly clotted), in thin preparations in the test tube, and to compare this with the effect of trypsin, collagenase, and papain. In these circumstances we have found no effect of Arvin in the test tube and we have used various pHs and molarities. The trypsin, collagenase, and papain all affected the tissues.

DR. R. GRAHAME (*London*) How did Dr. Holt measure the tensile strength and did he estimate the strength of healthy skin of the animal as well as that of the scar tissue?

DR. HOLT Measurement of healthy skin is very difficult and needs a large machine. We had thought about this.

The measurement of the tensile strength of the wounds is done by a Sandberg apparatus. It has prongs on either side and you just pull it apart, the tension being increased at a regular rate until the wound gives. It has quite a sharp endpoint which seems to be reproducible in an animal. In other words, the three duplicate wounds give similar results. If there had been a technical failure it would be expected to give a low result, so we took the two highest readings for tensile strength in both the control and the treated animals.

DR. B. MCCONKEY (Birmingham) What was the histological evidence that the normal skin was affected in the animals treated with Arvin? I am not sure if Dr. Holt mentioned the stain. Would he like to comment on this and the implications of the different staining characteristics?

DR. HOLT The stains were Van Gieson and Martius scarlet blue. I think the significance of the staining reactions is doubtful, and I attach no importance to them. The fibre sizes, I think, are definite. If my photography was good enough, I should have liked to show the polarized light slides which show the fibres very nicely indeed—the quite different size of the collagen fibres in the two groups in the normal skin. The normal skin shows two other characteristics which I have not illustrated: one is that the dermis is about two-thirds as thick in the treated animals as in the controls; the other is an abnormal regrowth of hair. This looks like hair of a newborn rabbit (say in the first 2 months of life), and is more fluffy than that of an older animal.

DR. A. S. RUSSELL (Taplow) From what Dr. Holt was saying about the antibody response to administered Arvin in the course of time, it seems to me that this is an elegant model of complex-induced disease, particularly at the equilibrium point. Did he notice any arteritis in the superficial vessels which might have some relevance, and did he notice whether defibrination had any effect on the development of fibrin deposits in the afferent arterioles of the kidney?

DR. HOLT There is a fibrin deposit, but it is very little and unlikely to cause trouble in the period during which one is able to give Arvin. As to whether these animals had any disease, all I can say is that I produced some hyperimmune animals for another purpose and they have all died. The *post mortem* examinations have shown nothing specific, but we are doing a chronic toxicity study on this basis. It seems unlikely that we shall evolve an immune complex disease because the amount of protein we are using is so small, a microgramme is one unit and to defibrinogenate an animal intravenously we usually use one unit per kg.—a minute amount. For persistent defibrinogenation we use something like 5 units daily intravenously or about 10 units twice daily intramuscularly, but these are still minute amounts of protein. Furthermore, no fall in complement has been shown.

References

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Effect of Arvin on Experimental Immune Arthritis in Rabbits. By P. M. FORD, F. W. S. WEBB, R. H. BLUESTONE, J. M. GUMPEL, and W. R. BELL (*Department of Medicine, Royal Postgraduate Medical School*)

A component of the inflammatory exudate had been suggested as the significant factor in the production and perpetuation of chronic immune arthritis in rabbits (Phillips, Kaklamanis, and Glynn, 1966). Fibrin has been strongly considered for this role.

Using Arvin (an extract of the venom of the Malayan pit viper) to reduce the amount of fibrin available for deposition in the region of the initial inflammatory reactions, we have sought to determine whether the subsequent arthritis could be modified in either severity or chronicity. Arvin acts by precipitating fibrinogen in the blood in the form of microclots and fibrin split products.

Intravenous Arvin was administered immediately before the introduction of antigen into the knees of previously immunized rabbits. Defibrinogenation was maintained for varying periods up to one month, the animals being killed at the end of the course of treatment.

Frequent measurements of joint diameter and histological examination after death showed essentially no difference between treated and control groups.

It is concluded, therefore, that, in this experimental model of immune arthritis in rabbits, Arvin has no effect on either the severity of the lesion or the establishment of the chronic inflammatory state.

Discussion

DR. P. A. BACON (London) Did the authors skin-test their animals? Suppression or diminution of both Arthus and delayed skin-reactivity has been observed using Heparin and it would be interesting to know whether this occurred with Arvin.

DR. FORD The animals were skin-tested only before intraarticular injection and not at the end of the Arvin treatment.

DR. B. MCCONKEY (Birmingham) Did the fibrinogen response to the injection in the untreated animals show a great variation or was it about the same magnitude for the same sized insult?

DR. FORD In the untreated control animals the mean fibrinogen level rose to 800 mg. per cent. and in fact all four animals showed a large rise to between 700 and 900 mg. per cent.

Reference

- PHILLIPS, J. M., KAKLAMANIS, P., AND GLYNN, L. E. (1966) *Ann. rheum. Dis.*, 25, 165.
see also FORD, P. M., BELL, W. R., BLUESTONE, R., GUMPEL, J. M., AND WEBB, F. W. S. (1970) *Brit. J. exp. Path.*, 51, 81.

Comparison of a New Latex Slide Test with the Sensitized Sheep Cell Test. By C. J. EASTMOND, D. PHILLIPS, and W. R. M. ALEXANDER (*The Rheumatic Diseases Unit, Northern General Hospital, Edinburgh*)

A new commercial slide test for rheumatoid factor was compared with the standard sheep cell test (SSCT) used in this unit. Two series of tests were made by two individuals: one amateur (CJE) and one professional (DP).