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 which 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
Dis., 25, 120.

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 defibrino-
genation on wound healing and connective tissue forma-
tion. 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 un-
touched skin were also found.

To elucidate the nature of these changes, in particular
the part played by Arvin directly and indirectly in de-
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 prepared through
paraffin. Engfeldt and Hjertquist (1967, 1968) have
demonstrated by the use of $^{35}S$O$_4$ that enormous losses
of matrix sulphated glycosaminoglycan quickly succeed
fixation in formaldehyde or glutaraldehyde followed by
postsomification and dehydration. In such preparations,
up to 70 per cent. of $^{35}S$O$_4$ 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 pro-
duced 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 defibrino-
genated. 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 extrac-
tions, but, judging the weight alone, we think it is follow-
ing 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
cotted), 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 efferent 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.

**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**


**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).