of infection with parainfluenza viruses after a long lapse of time. Studies over a lengthy period in Washington, D.C. (Parrott *et al.*, 1962), have revealed that parainfluenza viruses were prevalent throughout the year. Our study agrees with Ferris (1960) and Higgins *et al.* (1963) in finding a seasonal distribution of parainfluenza infection.

In Cambridge only febrile respiratory infection severe enough to require medical attention was investigated, and thus it was not possible to ascertain how much milder illness was due to parainfluenza viruses. Our survey has been carried out over a limited period of time and it is not certain that in other places similar findings will occur ; only prolonged and continuous observations of this kind can provide such information.

Summary

A survey conducted from September 1962 to August 1963 in two general practices in Cambridge showed that parainfluenza viruses were responsible for 18% of all acute respiratory infections investigated. There was evidence of parainfluenza virus infection in 35 patients, most infections being due to parainfluenza 1, but a few cases of parainfluenza 2 and 3 infection also occurred.

Croup was the commonest clinical manifestation in children. Infants and young children presented with the more severe illness, some degree of laryngeal obstruction frequently occurring. The older children presented with a milder illness, cough and hoarse voice often being found. There were 11 cases of parainfluenza infection in adults, most of whom presented clinically with an influenza-like illness.

The majority of cases occurred from September 1962 to January 1963. Studies of family episodes revealed that the highest secondary attack rate occurred in the youngest age-groups, and that the serial interval between cases was in the order of five days.

Viruses were isolated more easily from children than from adults, evidence of adult infection therefore being mainly serological. The complement-fixation test was more efficient than the haemagglutination inhibition test in detecting significant rise in antibody titre to the parainfluenza viruses, but the best results were obtained when both tests were employed.

We are indebted to a number of persons for their contributions to this study: Dr. A. P. Waterson for his help throughout the preparation of this paper ; Dr. R. B. Heath for doing the H.I. tests ; to Mrs. I. Nitkin for technical assistance, and Dr. C. M. Bradstreet, of the Central Public Health Laboratory, Colindale, for supplying serological reagents. We thank the Polio Research Fund for their generous contribution, which made it possible for this survey to be carried out.

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Inoculation of Volunteers with H Rhinoviruses*

D. TAYLOR-ROBINSON, M.D.; M. L. BYNOE, M.B., D.T.M.&H.

Brit. med. J., 1964, **1**, 540-544

The inoculation of human adult volunteers with an M rhinovirus, H.G.P., was reported by Bynoe *et al.* (1961). They showed that some volunteers developed colds and that most of these had low levels of serum antibody or no antibody before inoculation. On the other hand, many of the volunteers who did not develop colds had high levels of antibody before inoculation ; in other words, antibody had a protective effect. Similar results (Tyrrell, 1963) were obtained with another M rhinovirus, B632. M rhinoviruses grow in monkey-kidney cells in addition to human embryonic tissue and human malignant cells. Those rhinoviruses that grow only in human embryonic tissues and human malignant cells are termed "H viruses." They have been isolated more frequently than M viruses from subjects with colds (Tyrrell and Bynoe, 1961 ; Hamre and Procknow, 1961 ; Hamparian *et al.*, 1961 ; Johnson *et al.*, 1962 ; Kendall *et al.*, 1962), and the antibody response of children and adults to primary infection with H viruses is poorer than the response to M viruses (Taylor-Robinson *et al.*, 1963). Tyrrell and Bynoe (1961) demonstrated that some H rhinoviruses produced colds in volunteers, but they did not study the development of serum neutralizing antibody. Taylor-Robinson and Tyrrell (1962a) found that four of these H viruses were serologically distinct.

In the present experiments, viruses belonging to three of these serotypes have been inoculated intranasally into volunteers. Virus re-isolation in different human embryonic cells has been studied and serum neutralizing antibodies have been measured before and after volunteer inoculation in order to determine whether antibody prevents infection and/or colds.

Materials and Methods

Viruses.—The following three H serotypes were used: Sal./1/58H (F.E.B.), Sheffield/1/60H (16/60), and Sal./1/59H (Th.). A strain (T.) of the F.E.B. serotype and a strain (P.) of

* From the W.H.O. International Reference Centre for Respiratory Virus Diseases and the M.R.C. Common Cold Research Unit, Salisbury, Wilts.

the 16/60 serotype were inoculated, and were obtained from Dr. M. S. Pereira as nasal swabs in Hanks's saline containing 0.5% lactalbumin hydrolysate (L.A.H.) and 0.02% sodium bicarbonate. These materials had originally been collected from R.A.F. personnel in 1960 (Pereira *et al.*, 1963). Pooled nasal washings in Hanks's saline, from two volunteers (16 and 18) who developed colds after inoculation of the above T. strain, were used for further volunteer inoculations. Similarly the Th. virus was administered as a pool of nasal washings in Hanks's saline taken from two volunteers with colds which had been produced by inoculation of Th. virus. All viruses were stored with 1% bovine plasma albumin at −70° C. until used.

Tissue Cultures.—Primary trypsin-dispersed cultures of human embryo kidney (H.E.K.) cells were grown in a medium which contained 5% inactivated calf serum, 0.5% L.A.H., and Hanks's saline with 0.03% sodium bicarbonate and antibiotics; they were maintained in medium which contained 2% calf serum and 0.25% L.A.H. Batches of a semi-continuous strain of human embryo lung (H.E.L.) diploid fibroblasts were obtained from Dr. S. E. Smith (Wellcome Research Laboratories), and are termed B.W. cells in this paper. The human diploid-cell strain, WI-38, was obtained from Dr. J. P. Jacobs (M.R.C. Laboratories, Holly Hill, Hampstead). These H.E.L. cell strains were grown in 10% inactivated calf serum and Eagle's medium with antibiotics and maintained in Eagle's medium with 2% calf serum.

Volunteers.—Volunteers of both sexes, between 18 and 50 years of age, were isolated as described elsewhere (Andrewes, 1948), usually in pairs but occasionally singly or in groups of three. They were grouped at random, and until the end of the trial neither they nor the clinical observer were aware of the nature of the material given. After an observation period of three and a half days virus material was diluted tenfold in chilled Hanks's saline and was given as nasal drops in a volume of 1 ml. Blood was collected one or two days prior to virus inoculation; second blood samples were obtained 14 days after inoculation of Th. virus and about 21 days after inoculation of the T. and P. strains of virus. Nasal washings were collected in chilled Hanks's saline and inoculated direct into cultures or stored, with the addition of 1% bovine plasma albumin, at −70° C. and inoculated later.

Neutralization Tests.—Antibodies were measured by a microplaque reduction method (Taylor-Robinson and Tyrrell, 1962b) in which virus-serum mixtures were incubated at room temperature for two hours before inoculation into cultures of H.E.L. fibroblasts. The viruses used in these neutralization tests had been passaged several times through H.E.K. or H.E.L. cultures. All sera were inactivated at 56° C. for 30 minutes before use.

Inoculation of Volunteers and Clinical Illnesses Produced

An attempt was made to measure the amount of virus that was given to each volunteer, but this proved very difficult. Th. and T. strains of virus, present in the washings given intranasally, were titrated in primary cultures of H.E.K. cells. The Th. virus inoculum was not infectious for two different batches of cultures, so that each volunteer received less than one $TCID_{50}$; however, nasal washings from volunteers who developed colds after inoculation of the Th. washing yielded virus which was neutralized by a rabbit antiserum to Th. virus. T. virus in washings was titrated in a batch of cultures and this indicated that each volunteer received about 300–800 $TCID_{50}$ of virus. The great variation in the sensitivity of H.E.K. cells derived from different embryos was demonstrated by the fact that a repeat titration in cells from another embryo suggested that the volunteers had been inoculated with only about 10 $TCID_{50}$ of virus. The P. virus was not titrated, but it seems unlikely that the volunteer inoculum contained more virus than the T. virus inoculum.

Although apparently different tissue-culture doses of the viruses were given to the volunteers, colds developed in 4 out of 14 volunteers who were given Th. virus, in 4 out of 8 given P. virus, and in 11 out of 24 given T. virus. Two other volunteers had clinical signs of upper respiratory infection—for example, nasal stuffiness—but without coryza, and according to the criteria used at this unit these illnesses were not regarded as colds; and three more volunteers developed colds after the period of strict isolation. In all, 19 out of 46 volunteers who were inoculated with virus developed a definite cold during the period of isolation at the unit; 2 out of 29 volunteers who were in control groups and who were given Hanks's saline also developed symptoms of a cold. The colds produced by inoculation of these three H rhinoviruses were typical "common colds" with coryza, mucoid or mucopurulent nasal discharge, nasal obstruction, sore throat in some cases, and little or no pyrexia. The incubation period for the Th. virus strain was between 2 and 3 (average 2.75) days; for the P. strain between 3 and 4 (average 3.75) days; and for the T. strain between 1 and 3 (average 2.77) days.

Re-isolation of Viruses

Comparison of Tissue-culture Systems

Nasal washings were taken from all volunteers on the third and fifth days after inoculation and sometimes on the fourth day, whether or not they developed colds. All washings were inoculated in 0.3-ml. amounts into primary cultures of H.E.K. cells; at the same time washings from volunteers who had been given P. and T. virus strains were inoculated also into H.E.L. cell strains (B.W. and WI-38). The results are presented in Table I. The P. virus strain was isolated from each of four washings which were tested in H.E.K. cultures and from each of the same washings when they were tested in H.E.L. cultures whether or not the washings were inoculated direct or inoculated after storage at −70° C.; on the other hand, the T. virus strain was isolated from 11 out of 11 washings which were tested in H.E.K. cultures but from only 6 out of 8 and from none out of 8 of the same washings when they were tested in H.E.L. (B.W.) and H.E.L. (WI-38) cultures, respectively. Thus all the cultures seemed equally effective in detecting the P. virus strain, but the particular H.E.K. cells that were used were more sensitive than H.E.L. cells for the detection of the T. virus strain, and H.E.L. (B.W.) were more sensitive than H.E.L. (WI-38). It seems likely that different strains of H.E.L. cells vary in their virus susceptibility.

TABLE I.—*Comparative Virus Isolations in H.E.K. and H.E.L. Cells*

Virus given to Volunteers	Colds	Virus Isolation in		
		H.E.K.	H.E.L. (B.W.)	H.E.L. (WI-38)
P.	Yes	4/4*	4/4	4/4
T.	,,	11/11	6/8	0/8
Either P. or T. ..	No	0/5	0/5	0/5

* The numerator denotes the number of volunteers from whom virus was isolated and the denominator the number from whom attempts to isolate virus were made.

Correlation Between Re-isolation of Virus and Presence of a Cold

The results recorded in Table II show that virus was re-isolated from all 19 volunteers who developed colds and from only 6 of 27 volunteers who did not have colds. Of these six volunteers, two had clinical evidence of an upper respiratory infection but not colds, and two developed colds just after the period of strict isolation. Thus there were far fewer viruses isolated from volunteers without colds than from volunteers with colds. The difference observed is highly significant (χ^2 test with Yates's correction: $P<0.001$), and suggests that the colds that were observed in the volunteers were due to the viruses given to them.

TABLE II.—*Virus Recovery from Volunteers and their Antibody Responses*

Virus Given	Volunteers with Colds				Volunteers Without Colds			
	Virus Recovery	Antibody Response	G.M.‡ of Neutralizing Activity (K) of Sera		Virus Recovery	Antibody Response	G.M. of Neutralizing Activity (K) of Sera	
			Before Inoc.	After Inoc.			Before Inoc.	After Inoc.
Th.	4/4*	1/4†	0·02	0·2	2/10	1/10	0·1	0·13
P. ..	4/4	2/4	0·3	1·3	1/4	1/4	1·6	2·5
T. ..	11/11	7/10	0·06	1·4	3/13	7/11	1·4	3·7
	19/19	10/18	0·1	1·1	6§ 27	9/25	0·96	2·1

* The numerator denotes the number of volunteers from whom virus was isolated and the denominator the number who were inoculated with virus.
† The numerator denotes the number of paired sera showing an antibody rise and the denominator the number of paired sera tested.
‡ G.M. = Geometric mean.
§ Two of these volunteers had clinical evidence of infection but not colds, and two others developed colds after the period of strict isolation.

Neutralizing Antibody Studies

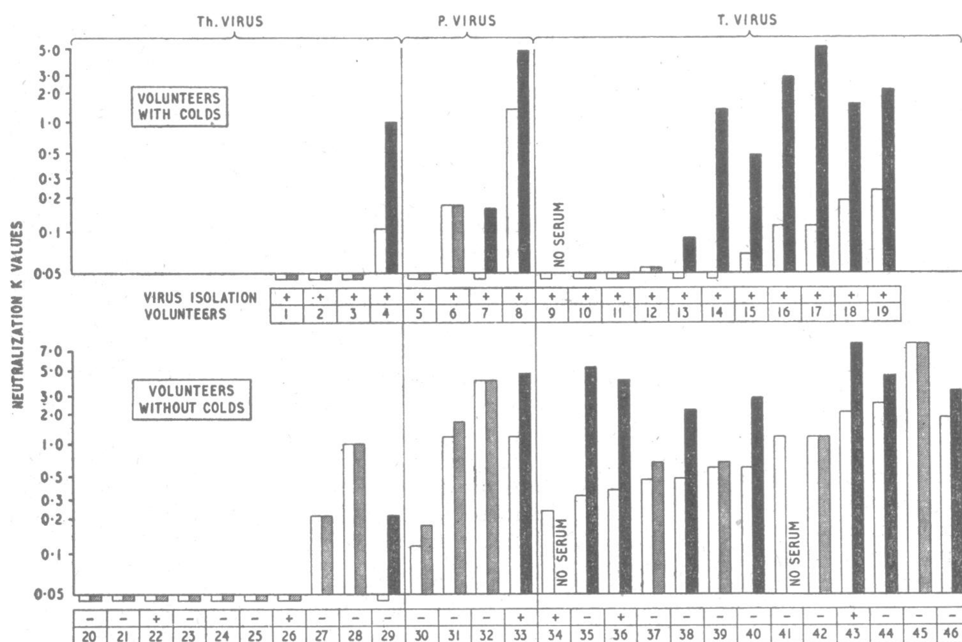
(See Table II and Chart.)

Volunteers with Colds

Antibody was not detected in 10 of the 19 preinoculation sera when they were tested at a 1/4 dilution, and very low levels of antibody (serum neutralizing activity K=0.1 or less), detectable only by plaque reduction, were found in five sera. The geometric mean (G.M.) of the serum neutralizing activity (K) against Th. virus was 0.02, against the P virus strain 0.3, and against the T. strain 0.06 ; the G.M. for all three viruses was 0.1. Thus, in general, those volunteers who developed colds had a low concentration of antibody in the initial serum specimen.

Eight of the post-inoculation sera showed no change in antibody titre (one serum was not available), while a rise in antibody titre was shown to have occurred in 10 sera ; the response was due to Th. virus in one instance, to the P. virus strain in two instances, and to the T. strain in seven. Some of these responses were small—for example, K<0.05 to 0.13—and others were larger—for example, K 0.1 to 5.2. The G.M. of the serum activity against Th. virus was 0.2, against the P. virus strain 1.3, and against the T. strain 1.4 ; the G.M. for all three viruses was 1.1—that is, tenfold greater than that of the preinoculation sera.

Further examination of the data in the Chart shows that of the eight volunteers who did not have an antibody response after inoculation of the H viruses, six had no detectable antibody in the first serum specimen, and of the 10 who did have an antibody response seven had antibody, although in low concentration, in the first serum specimen. Thus those volunteers who had a low concentration of antibody responded better than those who had no antibody at all—a " booster " response.



Antibody studies on sera from 46 volunteers and the viruses isolated from them after inoculation of H rhinoviruses Th., P., and T. □=Pre-inoculation serum. ▒=Post-inoculation serum (no antibody rise). ■=Post-inoculation serum (antibody rise).

Volunteers without Colds

Homologous antibody was detected in all but eight preinoculation sera of 27 subjects who did not develop colds ; all eight were from the 10 volunteers who were given Th. virus. Antibody was present at the time of inoculation in the sera of all four volunteers who were given the P. virus strain and 13 who were given the T. strain and who did not develop colds. The G.M. of the serum activity against Th. virus was 0.1, against the P. virus strain 1.6, and against the T. strain 1.4 ; the G.M. for all three viruses was 0.96. It is interesting that this G.M. figure for the activity of preinoculation serum of volunteers without colds is about the same as the G.M. figure for the activity of post-inoculation serum of volunteers who developed colds (Table II).

Closer examination of the data in the Chart shows that, apart from those volunteers who were given the Th. virus, 16 out of 17 volunteers without colds had a neutralizing activity of 0.2 or greater in the initial serum specimen whereas only 2 out of 15 with colds had such activity in the initial specimen. This difference is highly significant (χ^2 test with Yates's correction: $P<0.001$) and suggests that such serum neutralizing activity has prevented the development of the symptoms of a cold with the P. and T. virus strains.

Twelve of the 27 volunteers without colds had laboratory evidence of infection as judged either by virus re-isolation or by an antibody response. Sixteen of the post-inoculation sera showed no change in antibody titre (two sera were not available), but a rise in antibody titre was shown to have occurred in nine sera ; the response was due to Th. virus in one instance, to the P. virus strain in one, and to the T. strain in seven instances. All these antibody rises were threefold or more, except in two cases in which the rises were only twofold ; in these two cases repeat titrations produced the same results, and it seems likely that these responses are significant. The G.M. of the serum activity against the Th. virus was 0.13, against the P. virus strain 2.5, and against the T. strain 3.7 ; the G.M. for all three viruses was 2.1—that is, twofold greater than the preinoculation value. Thus in several instances, although there were no symptoms of a cold, infection occurred even in the presence of antibody, since there was a further increase in the antibody titre.

Discussion

It was not difficult to classify volunteers into those with or without colds. The distinction was usually clear-cut, even

though the colds were mild, because generally those volunteers who did not have colds did not have any symptoms ; only two volunteers had signs of clinical infection but insufficient coryza to warrant classification as colds.

The success in isolating viruses from all volunteers with colds and from a few without colds was probably due to the fact that the specimens were obtained and tested under ideal conditions. Nasal washings were taken at a time when symptoms had not long been evident or were most severe, and these specimens were dealt with within a few hours of being taken. The direct inoculation of cultures without prior freezing and storage of nasal washings does not seem to have been an important factor in the high isolation rate, since virus was re-isolated from aliquots of washings that had been stored at −70°C.

Comparison of different cells for the isolation of viruses showed that the P. virus strain was isolated successfully in all the cultures but that isolation of the T. virus strain was most successful in the H.E.K. cells that were used. T. virus was isolated in H.E.L. (B.W.) but not in H.E.L. (WI-38), which suggested a difference in virus susceptibility between these diploid-cell strains. Virus isolations were made in H.E.L. cultures maintained in a medium with an initial *p*H of 7.3–7.5 ; at this *p*H the cells did not show non-specific degenerative changes as rapidly as cells maintained in medium with a lower initial *p*H. It was important to observe both H.E.K. and H.E.L. cultures daily for cytopathic changes, since foci of degeneration in some cases regressed instead of progressing to involve the whole cell sheet.

Virus was isolated from all 19 volunteers who developed colds, but from only 6 of 27 who did not have colds, and this suggests that the viruses inoculated produced the colds that were observed. In general, those subjects who developed colds had little or no antibody prior to the inoculation of the H viruses. This is a finding similar to that of Bynoe *et al.* (1961), who worked with the M rhinovirus, H.G.P. However, they found that antibody rises occurred in 14 out of 15 of their volunteers with colds, whereas in the present study only 10 out of 18 volunteers had a rise in antibody concentration. Further comparison reveals that 9 of the 14 volunteers who had an antibody rise after inoculation of H.G.P. had final serum K values of >3.0 whereas only 2 of the 10 volunteers who had an antibody rise after inoculation of the H viruses had such final serum K values. This, perhaps, is not surprising, since Taylor-Robinson *et al.* (1963) found that the antibody response of subjects to natural primary infection with H viruses was poorer than the response to infection with M viruses. Thus it seems that H viruses produce antibody responses less frequently than M viruses, and when they do the antibody levels attained are not as high.

Only a few of the volunteers who were given Th. virus had pre-existing antibody, and antibody was not detected in the first serum specimen of 8 of the 10 volunteers who did not develop colds. Why, therefore, did they not develop colds? The amount of Th. virus, in terms of tissue-culture doses, that was given to these volunteers was very small, and it is possible that sufficient antibody was present in concentrations not detectable by our test, and that this protected against infection. However, this explanation seems unlikely, since one volunteer (No. 4) developed a cold although he had a low but detectable level of antibody at the time of inoculation. It seems more likely that the absence of colds in others might be due to the absence of infectious virus from the drops they received or to a non-specific resistance of their cells, analogous to the resistance of cells obtained from certain human embryos.

In contrast to the results with the Th. virus, homologous antibody was detected in the preinoculation sera of all those volunteers who failed to develop colds after being inoculated with the P. and T. virus strains. In fact, a serum neutralizing activity of 0.2 or greater apparently prevented the development of colds in 16 out of 17 subjects, a finding similar to that of Bynoe *et al.* (1961), who used H.G.P. virus. Moreover, Tyrrell *et al.* (1962) found that four volunteers who developed colds after inoculation of the H rhinovirus, D.C., had low levels of antibody, while four out of six who did not get colds had high levels. We conclude that circulating antibody may protect against disease produced by at least some of the H rhinoviruses and that vaccination might be an effective means of prevention. Mufson *et al.* (1963) have drawn similar conclusions from their work with an M and an H rhinovirus. These results are rather different from those of natural and experimental infections with parainfluenza and respiratory syncytial viruses in which illnesses occur in the presence of quite high concentrations of neutralizing antibodies (Reichelderfer *et al.*, 1958 ; Tyrrell *et al.*, 1959 ; Chanock *et al.*, 1961, 1962 ; Taylor-Robinson and Bynoe, 1963).

We were surprised that 7 out of 11 volunteers who did not develop colds after inoculation of the T. virus strain had an antibody rise. It seems, therefore, that the antibody circulating at the time of virus inoculation prevented the development of colds but did not, in these instances, prevent infection, as judged by an antibody response. Such asymptomatic infections may play a significant part in maintaining antibody levels in older adults and explain why they have few colds.

Summary

Three H rhinoviruses (Th., P., and T.) contained in nasal washings were inoculated intranasally into a total of 46 adult volunteers. Nineteen developed colds and virus was recovered from them all. Homologous neutralizing antibody could not be detected in the preinoculation serum of 10 of the 19 volunteers and only low levels were found in eight of the remaining nine sera. There was a rise in antibody titre in the sera of 10 out of 18 volunteers after recovery from their colds. Twenty-seven volunteers did not develop colds. Ten of these had been inoculated with the Th. virus and only two of them had antibody before inoculation ; the fact that antibody did not seem to play a part in preventing colds caused by this virus is discussed. In contrast all those volunteers who were given the P. and T. virus strains had antibody before inoculation ; this indicated that antibody played a significant part in preventing colds after inoculation of these viruses.

We thank all the volunteers for taking part in these experiments, Dr. M. S. Pereira (Central Public Health Laboratory, Colindale, London) for sending us viruses, and Dr. S. E. Smith (Wellcome Research Laboratories, Beckenham, Kent) and Dr. J. P. Jacobs (M.R.C. Laboratories, Holly Hill, Hampstead, London) for the diploid fibroblast cell strains. We are grateful to Mrs. P. K. Brown and Miss B. Ridgwell for valuable technical assistance, and to Dr. D. A. J. Tyrrell for his help and advice throughout the course of this work.

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Medical Memoranda

Haemophilic Pseudotumour After Fractured Femur

[WITH SPECIAL PLATE]

Brit. med. J., 1964, **1**, 544

Haemophilic pseudotumour is an uncommon feature of a rare disease; about 40 cases are described in the literature, and in a survey of 109 cases of haemophilia and Christmas disease in the Birmingham area the following was the only example encountered. It is a huge tumour, and unusual in complicating a fractured femur.

Case Report

The patient, now aged 32, is the grandson of a haemophiliac. Haemophilia was suggested soon after birth by signs (which did not persist) consistent with intracranial haemorrhage, and has since been confirmed by the thromboplastin generation test. His childhood was typical of severe haemophilia, with repeated haemarthroses and episodes of haematemesis and haematuria. At 13 a haematoma in the right arm caused a Volkmann's contracture. At 17 mental confusion and a speech defect developed spontaneously; he improved after a while, but personality change, dysphasia, and epilepsy were permanent sequelae.

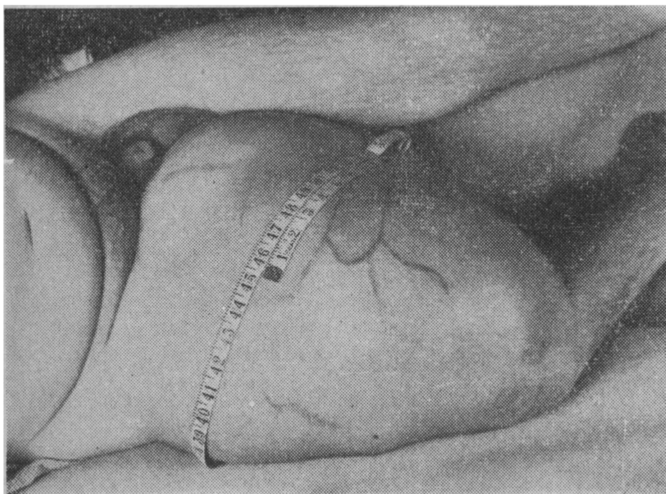

FIG. 1.—Haemophilic pseudotumour of right thigh.

When he was 24 he had a fit while sitting in a chair and sustained an oblique fracture of the femoral shaft. Treatment consisted of traction and several blood transfusions. Fourteen days later there was increased displacement of the bone ends radiologically and his leg was very swollen; traction was continued. Three months after the fracture the swelling was the same and there was no evidence of union. He became very discontented with hospital and was allowed home after four and a half months.

A year later his leg was even bigger and an *x*-ray film showed gross separation of the bone fragments. He refused to consider amputation on this occasion and several times afterwards. The tumour has continued to grow slowly and he has been persistently anaemic. The right leg is now (Fig. 1) 45 in. (114 cm.) in circumference and radiologically (Special Plate, Fig. 2) there is extensive destruction as well as separation of the pieces of femur. The knee-joint shows the changes of haemophilic arthropathy: the hip-joint and pelvis have been spared.

Discussion

Abell and Bailey (1960) reviewed 17 cases of haemophilic pesudotumour from the literature, and Fraenkel, Taylor, and Richards (1959), using the term haemophilic blood cyst, discussed eight more; each paper contributed two case reports. Horwitz, Simon, and Bassen (1959), Birk (1960), Crock and Boni (1960), Schwarz (1960), and Nelson and Mitchell (1962) have described further examples. The common feature seems to be bone involvement, usually of the ilium or femur. Association with fracture is not often mentioned; Ghormley and Clegg (1948) described two such cases and Egeberg, Borchgrevink, and Hjort (1960) another. Witts and Allison (1960) suggested that the initial lesion was usually a subperiosteal haematoma. Jordan (1958) stated that in most haemophiliacs fractures heal quickly and well, but the present case suggests that if there is failure of union pseudotumour may be inevitable.

Until recently the great dangers of surgical intervention, in particular of aspiration biopsy, have been stressed. Egeberg *et al.* (1960) and Hall, Handley, and Webster (1962), however, have now reported successful surgery for pseudotumour under cover of parenterally given antihaemophilic globulin; two leg amputations are described and one excision of tumour. Initially amputation would have been the treatment of choice in the present case had the patient been willing, but his condition is now such that the technical difficulty and the risk of operating are considered unjustified.

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J. F. HARRISON, M.B., M.R.C.P.,
Medical Registrar, the General Hospital, Birmingham.

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