A CHANGE IN RABBIT FIBROMA VIRUS SUGGESTING MUTATION

I. EXPERIMENTS ON DOMESTIC RABBITS

BY C. H. ANDREWES, M.D.

(From the National Institute for Medical Research, Hampstead, London)

PLATE 14

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In 1932 Shope (1) described an infectious fibroma of the cottontail rabbit. This proved to be caused by a filtrable virus which would infect not only cottontails but also domestic rabbits. On inoculation into the testis, skin or muscle, the virus produced localized tumour-like swellings, apparently formed from multiplication of young connective tissue cells. The growths did not metastasize and ultimately always regressed, more quickly in the domestic rabbit than in the cottontail. Few, if any, pathologists would regard them as true tumours.

In this paper I have described observations made in England on the behaviour of strains of virus sent to me by Shope. He has recorded, in the succeeding paper (2), the results of parallel observations made by himself at Princeton. In the third paper of the series (3), we have jointly discussed the interpretation of our findings. A certain amount of repetition and cross-reference in the first two papers is unavoidable.

In February, 1933, I received from Shope glycerolated material from two strains of fibroma virus, A and B. One of them, Strain A, was that studied in Shope's original experiments; yet it behaved from the moment I received it in a manner quite different from that described by him. It no longer gave rise to fibroma-like growths, but produced only acute inflammatory lesions; furthermore, many inoculated rabbits developed a generalised pock-like eruption on the skin. In the succeeding paper (2), Shope describes the history of the A strain of virus before he supplied me with material, and also records a similar but less extensive change which the virus underwent in his laboratory.
In November, 1933, Shope kindly gave me material of his A strain which had been preserved in glycerol for 18 months and dated back to a time before the first appearance of any inflammatory traits. In my hands this strain has continued on passage to produce fibroma-like lesions such as Shope originally described. I have thus been able to compare the two strains side by side; one is referred to as the original A (OA), the other as the inflammatory A (IA) strain. Evidence will be presented that the two cross-immunise, one against the other, and that the IA does not represent a contamination with any other known virus.

**Behaviour of the OA Strain**

The original A strain behaved, in my hands, mainly according to Shope's description (1). In rabbits killed a week or less after intratesticular inoculation, the naked eye appearance of the testis was that of an acute inflammation rather than tumour formation; there was scrotal oedema and the testis was engorged and exuded fluid on section. Later than this, the formation of large fibroma-like masses was more and more evident; these persisted for a week or two before regression. In some animals, however, the testis became impacted in the inguinal canal in the course of its enlargement and then haemorrhagic necrosis set in, instead of further increase in size.

In one respect my experiences differ from those described by Shope; subcutaneous inoculation gave inconstant results, but intradermal injection of 0.1 cc. of virus regularly produced sharply circumscribed raised red lesions. These appeared in 2 or 3 days and grew until about the 12th or 14th day, often reaching a diameter of 4 or 5 cm. and a thickness of more than 1 cm. The skin over them often had a vesiculated appearance. After 12 or 14 days regression began, with or without central necrosis of the skin overlying the lesion. Small satellite lesions were not infrequently seen. Suspensions of affected testes often produced skin lesions up to a dilution of 1:10,000 or 1:100,000 of the original 10 per cent testicular emulsion, and a convenient method of titrating the virus was thus available.

**Histology of OA Lesions**

Testes removed 3 to 6 days after inoculation often showed histologically hyperaemia, local oedema of the interstitial tissues and some exudation of polymorphonuclear cells. Later one could see that large mononuclear cells were present in increased numbers and soon the whole picture was dominated by the presence of large numbers of young fibroblast-like cells, showing numerous mitotic figures (see Fig. 1). The fibroma-like tissue thus formed continued to increase for some days and apparently to become more mature and less cellular as it did so. In the midst of this older fibromatous tissue, blood vessels could often be seen surrounded by a more cellular zone. The period at which these changes occurred
was inconstant; the dose of virus used was an important factor. Thus, testes removed after 7 days showed sometimes only the earlier exudative stage, but more usually the fibroblastic proliferation had already made considerable progress. In some testes the proliferative reaction was found to have set in within a few days of inoculation and no trace was evident that there had ever been any oedema or exudation of polymorphonuclear leucocytes. Except where local necrosis occurred, signs of inflammation regularly became less as the fibroblastic proliferation continued, but often oedema, hyperaemia and polymorphonuclears were evident even in the later lesions in the visceral layer of the tunica vaginalis, around the epididymis and in the fat in the spermatic cord. Necrosis of the germinal epithelium was often present a week after inoculation, but all the evidence suggests that it was secondary to the changes in the interstitial tissues.

Histological studies of the skin lesions were less extensive. The most prominent feature at first was the oedema of the subepidermal fibrous tissue; mononuclear infiltration occurred around the hair follicles and the deeper blood vessels. Later, fibroblastic proliferation began in the oedematous connective tissue near these vessels outside the panniculus carnosus. The fully developed growths both in skin and testis showed the changes described by Shope (1), being almost entirely composed of masses of fibroblast-like cells arranged in sheets, strands or whorls.

Behaviour of the IA Strain

The early changes produced by the two strains of virus are apparently identical. A few days after intratesticular inoculation with the IA strain the testes on palpation are full and firm and there may or may not be scrotal oedema. When testes from rabbits killed less than a week after inoculation are examined, it is often impossible to tell with the naked eye which strain is concerned: in both instances there is evidence of acute orchitis. Similarly the skin lesions produced by the IA strain are at first like those already described. Rarely, however, are they as definitely raised and circumscribed as the OA lesions. It is in the later behaviour of its testicular and skin lesions, that the IA strain shows itself to be so different. After a week the acute orchitis subsides, but the testis, instead of progressively enlarging, rapidly begins to atrophy and a fortnight from inoculation it is already much smaller than normal. The raised red areas on the skin also begin to decrease in size on the 6th or 7th day and central necrosis is then the rule. The skin lesions produced by both strains have been measured daily and the changes in their size plotted graphically; it has then been very striking to see how those of the OA virus continue to grow after the 6th or 7th day, though more slowly than at first, while those of the IA strain rapidly resolve and have often disappeared at a time when comparable OA skin growths are still progressing.

Histology of IA Lesions

Histologically, also, the two strains produce at first similar changes. But with the IA virus the early oedema and polymorphonuclear infiltration soon give
place to massive accumulation of lymphocytes in the interstitial tissues (Fig. 2). This is accompanied by atrophy of the parenchyma. After a week fibrosis and atrophy of the testis as a whole supervene. The fibrosis is such as will follow almost any acute orchitis and cannot readily be confused with the vast fibromatous proliferation produced by the other strain. In the early stages a number of large cells, possibly histiocytes, are sometimes seen in the testicular interstitial tissues, apparently dying as the result of the infection; they are swollen and have flattened crescentic nuclei pushed to the periphery of the cell.

The IA skin lesions are like those of the other type in all their early stages. The later fibromatous proliferation is, however, absent and the prompt regression of the lesion is accompanied by copious accumulation of lymphocytes.

**Generalised “Pocks” Produced by the Rabbit Fibroma Virus**

At the 6th serial rabbit passage after I had received from Shope the IA strain, I first noted the appearance in the skin of small red papules strongly suggestive of the early lesions of generalised vaccinia in the rabbit. Subsequently, between April, 1933, and October, 1934, the eruption was seen in 43 out of 80 inoculated rabbits (54 per cent) in which the hair had been clipped and the skin observed for 7 days or more. The rash appeared usually on the 6th, 7th or 8th day after inoculation. The papules were raised, red, 2 to 5 mm. in diameter, occasionally larger; they grew for a day or two at most and then regressed without vesiculation or pustulation. They appeared after intratesticular or intradermal inoculation and could be seen on the skin of a flank on which the hair had been clipped but which had not been itself inoculated. Papules were not seen on the ears or mucous membranes. Virus was recovered from the papules by excising them, grinding them up and inoculating a suspension into another rabbit. I failed to recover virus from areas of skin not showing papules, nor in two attempts did I obtain any from the blood of an infected rabbit. Not only was whole blood used in these two experiments, but also, in view of Smith’s work with vaccinia (4), washed blood cells.

Generalisation of the virus to the skin was at first thought to be peculiar to the IA strain of virus. Though it was not noted until the 6th passage of this strain at Hampstead, in only 2 rabbits of earlier passages had the hair been clipped; an eruption would thus probably have been unobserved. In experiments with the OA virus no generalisation was seen during the first 7 passages; at this stage 22 rabbits had been clipped and watched for the appearance of an eruption, with
negative results. Generalisation was first seen at the 11th passage and thereafter it appeared frequently. Otherwise, the virus behaved as before and showed no tendency to change to the IA type. When generalisation unexpectedly appeared in the OA series, I went back to glycerolated rabbit testis of the 6th, 8th and 9th passages, which had been kept in the cold room, and proceeded to make serial transfers from them. In 4 different series, skin eruptions appeared in rabbits inoculated with virus of 8th, 9th, 10th and 11th passage respectively. In 33 rabbits of the 7th and subsequent passages generalisation occurred in 9, or 27 per cent. The pocks were like those of the IA strain and showed, like them, no tendency to progressive increase in size. Histologically, also, the secondary lesions produced by the two strains were alike; there were no constant changes other than local oedema and round celled infiltration. Virus recovered from pocks of OA rabbits proved on further inoculation always to be of the OA type.

An unexplained feature of the generalisation is this: it has regularly occurred in my hands, with both strains and in rabbits of several different breeds including Dutch, Chinchilla, Himalayan, Belgian hares and half-lops; yet Shope (2) has never noted it with either virus strain in America, though he has watched for it carefully. He has worked with all the breeds of rabbits mentioned, except the half-lops. The conditions determining generalisation are thus obscure. As already mentioned, it occurred in 54 per cent of my rabbits over a period of 18 months. After that it was seen much less commonly, between October, 1934, and May, 1935, only in 12 out of 74 inoculated rabbits (16 per cent).

"Inclusion Bodies"

Shope (1) reported that the fibroma virus produced in the cotton-tail rabbit skin lesions recalling those of molluscum contagiosum in man. Masses of epithelial cells projected downwards and many cells were enlarged and contained eosinophilic masses in their cytoplasm. He found, however, no "inclusions" in the lesions in domestic rabbits. I, too, have failed in the large majority of sections of tame rabbit material examined to find any inclusion bodies, nor have I ever seen changes as striking as those found in cottontails. I have,
however, found in the cytoplasm of epithelial cells in two sections of skin, eosinophilic masses recalling the cottontail inclusions. One of these was in a rabbit infected with the IA strain, the other in an OA rabbit. In the epididymis, also, I have found on two occasions areas of considerable epithelial proliferation. In one rabbit, infected with OA virus 7 days before, this proliferation entirely occupied the lumen of many tubules; the epithelium was degenerate and inclusions could not be recognized with certainty. In the other rabbit, inoculated 10 days previously with the IA strain, the proliferation was less striking but eosinophilic inclusions were visible in the cytoplasm of many epithelial cells in the tubules. These few observations suggest, particularly in view of the changes it produces in the cottontail, that virus of both strains is potentially epitheliotropic in the domestic rabbit.

Cross-Immunity between OA and IA Strains

Rabbits recovered from infection with one strain were found to be immune to inoculations of the other. The existence of cross-immunity was shown by the absence of lesions on reinoculation into skin or testis or the occurrence at the site of an intradermal inoculation of an accelerated allergic type of response reaching its maximum in 48 hours. Accelerated reactions of this type occurred just as frequently when the test was made with the homologous strain as in cross-immunity tests. 17 rabbits inoculated into the skin with IA virus were immune when tested after 14 to 38 days with OA virus. 5 other IA rabbits were similarly immune when tested 7 to 37 days later with another strain of virus, B, which at that time was behaving in all respects like the OA strain. Conversely, 8 rabbits recovered from OA infections were found resistant to the IA virus in tests made after 14 to 24 days.

Neutralisation tests with immune rabbit sera confirmed this result. It was not even possible to demonstrate quantitative differences in neutralising power such as can be shown when antisera against various fowl tumour viruses are carefully compared with each other. Undiluted OA virus when mixed with an equal volume of 1 in 5 antiserum and held for an hour at room temperature was not neutralised either by homologous (OA) or by IA antiserum; 1 in 5 dilutions of both sera
were, however, effective against the same virus diluted 1 in 10. In tests against IA virus, neither serum in a 1 in 5 dilution was able to neutralise completely even the 1 in 100 dilution of virus; but undiluted sera from both OA- and IA-immune rabbits completely inactivated undiluted virus.

Tests for Cross-Immunity between IA Virus and Other Viruses

When first the IA virus began to behave in an unexpected manner and particularly when generalised pocks were seen, it was suspected that the material under study might have become contaminated with vaccinia or some other virus. It was found, however, that a rabbit recovered from infection with IA virus was, when tested 25 days later, fully susceptible to intradermal inoculation with neurovaccinia virus. Further, the IA virus was not in the smallest degree neutralised by an antivaccinial serum of proved activity.

Dr. Louise Pearce kindly gave me two sera from rabbits recovered from the rabbit pox studied at The Rockefeller Institute (5); neither had any neutralising power against IA virus. The virus was also unaffected by antiserum active against Virus III of Rivers and Tillett (6).

A few experiments were carried out bearing on a possible relationship between the rabbit fibroma and a true tumour of the rabbit. 14 rabbits in which Brown-Pearce tumours had regressed were taken; 7 of them were hyperimmunised with 1 to 6 subcutaneous injections of cells from the same tumour and then all were tested intradermally with fibroma virus (IA) along with control rabbits. The tumour-immune rabbits all proved fully susceptible. Sera obtained from 7 of the tumour rabbits before this intradermal test were found to be devoid of neutralising power for IA virus.

Four antisera active against fowl tumour viruses (Rous No. 1, Fujinami, RF4 and CT10) (7) were tested and found to be unable to neutralise IA virus. Experiments on cross-immunity with rabbit myxoma virus are reported in the following paper (2).

Attempts to Convert One Strain into the Other

The available evidence suggested that the IA strain was not a contamination with any other known virus, but that it and the OA
strain were varieties of one virus, possibly differing in their virulence for the cell. The following experiments were planned with this as a working hypothesis.

IA virus was passaged at approximately weekly intervals from testis to testis through 28 rabbits in series; it showed no tendency to revert to the fibroma-producing phase. After this, transfers were made at fortnightly intervals for 3 passages. Very little IA virus is recoverable from a testis after 14 days and it was thus hoped to attenuate it or decrease its virulence. Although virus was not readily carried on by passages at these longer intervals, no tendency was seen after 4 such transfers for the virus to produce fibromatous changes or otherwise change its character. An attempt was made to lessen the vigour of the reaction to the virus by lowering the temperature at which the reaction occurred: inoculations were accordingly made intradermally into the ear of a half-lop rabbit, but the type of lesion was not essentially modified.

Next, injections of IA virus were made into a rabbit's left testis a few days after a primary inoculation into its right testis; it was thought that if the cells should possess a small degree of active immunity they might react less violently and produce an OA type of lesion. This hope was not realised; when the interval between the injections was 2 days, the second inoculation produced a reaction of the usual type; the same was seen after a 4 day period except that lymphocytic infiltration was more than usually prominent. When the interval was extended to 6 days, the second testis was apparently immune, as it appeared normal microscopically. In yet other experiments, incomplete neutralisation of IA virus with antiserum failed to influence the type of reaction produced by it.

A similar lack of success attended efforts to enhance the virulence of the OA virus so as to cause it to produce only an inflammatory tissue response. After 14 rapid passages at approximately weekly intervals its behaviour was in this respect unchanged; it had, however, as already recorded, apparently acquired the power of producing a generalised skin eruption.

*Shope's “Changed Virus”*

In October, 1933, Shope gave me a glycerolated virus in rabbit testis with the following history: it was derived from virus which produced predominantly but not entirely inflammatory reactions and is described by him as changed virus (2); this, as he has recorded, was inoculated into a cottontail rabbit, and the virus recovered therefrom was found to have regained temporarily its fibroma-producing properties. When I inoculated this into rabbits, only fibromatous (OA) lesions were seen in the first animal infected, but passage from that rabbit's testis produced, in four rabbits, predominantly inflammatory
lesions. From the 2nd passage onwards, generalisation was frequent. Testes of 8 rabbits of the 2nd to 4th passages, when killed after 7 to 31 days, showed remarkable changes, strongly suggesting a mixture of the effects produced by the OA and IA strains. When such testes were sliced in half, firm white nodules could be seen in the midst of softer hyperaemic tissue. The impression gained was confirmed by histological study. Through the greater part of the testis could be seen severe inflammatory reaction in the interstitial tissues; in most sections lymphocytes predominated. In the midst of this inflamed tissue were sharply circumscribed fibromatous nodules, often with the proliferating cells arranged concentrically (see Fig. 3). It was difficult to avoid the conclusion that two different processes were simultaneously going on in the same testis. In none of the rabbits in this group did the testis become much enlarged; only one, however, was allowed to survive for more than 15 days. In later passages with this strain, a fairly intimate mixture of fibromatous and inflammatory lesions was frequently observed histologically. The results of passing the changed virus serially through rabbit testis are shown in Chart 1. Occasionally, as shown, virus was stored in glycerol in the cold before passages were continued. It will be seen how varied and unpredictable was the histological response in the rabbits' testes. Individual rabbits in the chart will be referred to presently.

**Attempted Separation of IA and OA from Changed Virus**

Acting on the supposition that this was a mixed strain containing both IA and OA viruses, I planned a number of experiments with a view to separating the two strains. First, virus was inoculated into the testis of a guinea pig, recovered 3 days later and passed again into rabbits; it was thought possible that one strain might survive better than the other in a foreign host. This idea was not supported, for the rabbit inoculated from the guinea pig testis showed lesions of mixed type. Secondly, rabbits were inoculated from changed virus diluted out to the limits of its infectivity. It was argued that if this virus really consisted of a mixture of two strains, it was unlikely that these were present in equal quantity; possibly, therefore, the inoculation of relatively few minimal infective doses might permit the recovery of one or other strain pure. In a first experiment, a rabbit (1-66, Chart 1) was inoculated intratesticularly with 0.001 cc. of "mixed" virus having a minimal infective dose for the skin of 0.0001 cc.; examination of its testis left no doubt that the strain was still mixed in character. A similar experiment was
CHART 1. Diagram of passages of changed virus through rabbit testes. A cross-hatched ellipse indicates a testis with fibromatous lesions. A dotted ellipse indicates a testis with inflammatory lesions. The nature of the lesions in other testes are suggested by various types of mixtures of cross-hatching and dotting. Figures at the sides of the ellipses are the serial numbers of the rabbits. The figures in circles indicate how many days passed between inoculation of the testis and killing the rabbit for passage or histological study. Small circles outside of some ellipses represent generalized pocks.
carried out with the B strain of virus which was at that time producing mixed lesions. A rabbit was inoculated into right and left testes with 0.01 cc. and 0.001 cc. of virus respectively. No lesions developed in the left testis, which received the smaller dose, nor in the testes of 2 rabbits inoculated in series therefrom. Small lesions appeared in the right testis, which thus presumably received less than 10 testis-infecting doses. Passages were made in series through 7 rabbits, and it was again clear that the virus was still mixed. I thus failed to recover a pure strain by this method, but my hope of succeeding perhaps rested on a fallacy; it is pure supposition that 1 minimal infective dose represents one virus particle; the small doses used for inoculation in these experiments may actually have contained several hundred virus bodies.

Next the attempt was made to recover pure OA virus by passing at long intervals. Shope recovered virus of the OA type from the testes of rabbits inoculated 21 and 35 days previously. The IA virus, however, proved in my hands difficult to isolate from testes injected a fortnight before. It was thought possible that the IA virus would in time disappear from a testis infected with the changed strain and passage was therefore made from a testis 3 weeks after inoculation (rabbit 5-01, Chart 1). The result was not what was expected, for the resulting lesions were predominantly inflammatory. The fourth method tried was the recovery of virus from one of the generalised pocks on the rabbit's skin. It was hoped to be able to pick out a pure "colony" conveniently "plated out" in this way. A rabbit (1-64, Chart 1) inoculated with changed virus developed only inflammatory lesions in the testis and also a generalised eruption on the skin. It has already been mentioned that in occasional rabbits inoculated with this strain only inflammatory lesions were seen, but that the mixed character of the strain was shown by the results of later passages. The rabbit was killed; several pocks were excised, ground with pyrex glass and saline and inoculated into the skin and testis of another rabbit (1-68). Typical lesions of IA type developed and from the infected testis virus was carried on in series. It was passed through 6 "generations" in all, involving 9 rabbits, and in none were any but inflammatory lesions seen. It seems fairly certain, therefore, that in this experiment a pure IA virus was recovered. At the time the experiment was carried out, generalisation had only been encountered in rabbits infected with IA virus. A year later, when both strains were liable to generalise, the experiment was repeated, but on this occasion the virus recovered from the excised pocks was still of mixed type (rabbits 4-67, 4-86, etc., Chart 1).

**Experiment with B Strain of Virus**

The B strain of virus, which has been briefly referred to before, was derived from a spontaneous growth in a different cottontail rabbit from that which originated the A strain. It has been studied by Shope and in England by my colleague, Dr. W. J. Purdy, and has regularly produced lesions of the fibromatous type. Dr. Purdy gave me two sealed ampoules of virus which had been dried; after receiving the virus from Shope, he had made only 2 rabbit passages before
Rabbits inoculated with the dried virus from one ampoule showed only fibromatous lesions in the first 2 generations; thereafter the lesions were mixed in character, exactly like those of Shope's changed virus. Virus from the other ampoule also produced fibromatous reactions in the first 2 rabbits of the series; the 3rd rabbit showed mixed lesions; the testes of the 4th were not examined histologically. Those of the 5th to 10th inoculated in series showed an entirely inflammatory response like that of the IA virus, except that there was a small area with an atypical fibroblastic reaction in the rete testis of the 7th transfer rabbit.

It seems probable that the B strain of virus has undergone a change similar to that described for the A strain. It is unlikely that it could have been contaminated by my A strain for the following reasons. First, two separate ampoules dried in Purdy's laboratory at Mill Hill, where there had never been any A virus, yielded mixed and in one instance possibly pure inflammatory virus, on passage through rabbits at Hampstead. Secondly, I can confirm for the IA strain Shope's (1) finding that with the OA virus cross-infection of one domestic rabbit from another does not occur under laboratory conditions; I found no evidence of infection even when young animals were kept together in one cage, and when the intranasal route of infection was used.

It must be noted that in Purdy's hands the B strain did not lose its fibroma-producing properties after 21 passages; he always took care, however, to pass from rabbits in which the lesions appeared to be proliferative in type.

Artificial Mixtures of Strains

It was decided to study the results of mixing together pure OA and pure IA viruses in various proportions and passing from the mixtures in series through rabbit testes. The results of testing 12 such mixtures are shown in Table I. When the mixture contained a very large excess of OA virus, no signs of the presence of IA strain were evident after 4 passages (Experiment 4). Similarly with a tenfold excess of IA virus (Experiments 7 and 8), the OA virus was not apparent after 3 passages. The results of passing another mixture (Experiment 2) are shown in more detail in Chart 2. No differences were detected between the behaviour of this artificially mixed strain and the supposed natural mixture discussed earlier (see Chart 1); many inoculated testes when examined histologically showed lesions mixed in character. In
occasional rabbits the lesions were apparently almost entirely fibromatous or almost entirely inflammatory, but the testes were not examined in serial sections. It was very remarkable that on several occasions a rabbit inoculated with emulsions of an almost purely fibromatous testis itself developed almost wholly inflammatory lesions. Much less commonly the reverse occurred, “inflammatory” testes giving rise at the next passage to fibromata. As with the supposed natural mixture, there was no regular tendency for the lesions

TABLE I

Results of Inoculating Artificial Mixtures

| Mixture | Minimal skin-infecting doses in mixture | Lesions in rabbit of | Total No. of passages |
|---------|-----------------------------------------|----------------------|-----------------------|
|         | IA                                      | OA                   | 1st passage | 2nd passage | 3rd passage |                        |
| 1       | (Virus diluted 1 in 2)                   | (Virus diluted 1 in 2)| OA         | Mixed       | Mostly IA   | 3                       |
| 2       | (Virus diluted 1 in 200)                 | (Virus diluted 1 in 2)| OA         | OA          | Mixed*      | 9                       |
| 3       | 500                                     | 5000                 | OA         |             |             | 1                       |
| 4       | 5                                       | 5000                 | IA         | OA          | OA†         | 4                       |
| 5       | 500,000                                 | 5000                 | IA         | IA          | IA          | 1                       |
| 6       | 5000                                    | 50                   | IA         |             |             | 1                       |
| 7       | 500,000                                 | 50,000               | IA         | IA          | IA          | 3                       |
| 8       | 5000                                    | 500                  | IA         | OA          |             | 3                       |
| 9       | 500,000                                 | 500,000              | Mixed      | Mixed       | IA          | 3                       |
| 10      | 5000                                    | 5000                 | Mixed      | Mixed       | IA          | 3                       |
| 11      | 500,000                                 | 50,000               | OA         | Mixed       | Mixed       | 3                       |
| 12      | 50,000                                  | 500                  | Mixed      | Mixed       | Mixed       | 3                       |

*Remained mixed in subsequent passages (cf. Chart 2).
†OA in 4th passage rabbit also.

in the series to become steadily more inflammatory or more fibromatous. One attempt was made to recover a pure virus from pocks on the skin of a rabbit receiving a mixture. This was carried out on a rabbit in Experiment 2 and the result is shown in Chart 2 (rabbits 1-40, 2-47); the virus recovered was, as in one of the experiments with the “natural mixture,” of mixed character still.

Irregularity of Rabbit Response

The behaviour of the virus in the changed and artificially mixed series is in striking contrast to that of the pure OA and pure IA viruses.
Diagram showing results of inoculating one virus mixture into different rabbits. For explanation see Chart 1. For most rabbits in this chart the histological responses in right and left testes are shown separately.
I have carried on the OA virus through 17 rabbits without detecting in it any tendency to change its character; the IA strain has similarly been passed serially through 32 rabbits and has at no time given rise to any fibromatous lesions. The recovery of pure IA virus from the changed virus and the exactly similar behaviour of the changed virus and artificial mixture make it seem almost certain that the changed virus is in fact a natural mixture. For a long time it seemed puzzling that the balance between the two constituents in the strain should have been maintained so evenly. The results of passing unequal mixtures (such as Nos. 4, 7 and 8 in Table I) suggest that if one strain came temporarily to preponderate, it might soon supplant the other completely. Doubtless this can happen at times and such an occurrence would explain the recovery of a pure IA strain from the virus originally sent me by Shope and later from the B strain of virus. But it is possible that irregularity in the response of rabbits to the mixture may ordinarily act as a stabilising factor. In this connection the experiment whose results are shown in Chart 3 is particularly interesting. Rabbit 5-23 was inoculated with OA and IA virus in equal parts and developed almost wholly inflammatory lesions in the testis. A centrifuged emulsion of this testis produced in 1 rabbit predominantly OA lesions; 4 other rabbits injected with the same virus after it had undergone a short stay in glycerol showed respectively wholly inflammatory, almost wholly inflammatory, mixed and wholly fibromatous lesions. A further passage from the last rabbit gave rise to lesions which were again very varied in character as the chart shows. All the rabbits received similar material into each testis and it was striking that the lesions in the right and left testes of each of the 8 rabbits were of the same nature. Thus any differences must have been attributable to something inherent in the individual rabbits and not to accidental factors. It is further clear that they were not due to qualitative differences in response to a single stimulus, but rather to the fact that some rabbits favoured the fibromatous virus in the mixture, others the inflammatory one. This must be so since either virus will regularly give rise to its own characteristic histological response when introduced by itself.

No attempt has been made to analyse the differences in rabbits which led to this result. But if rabbits favouring the IA virus and those favouring the OA were fairly equally distributed amongst the
stock used for passage, there would clearly be a tendency, normally, for a mixed virus to retain its mixed characters.

SUMMARY

A strain of rabbit fibroma is described causing in inoculated animals acute inflammatory lesions very different from the fibroma-like growths induced by the original strain. The new inflammatory strain cross-immunised with the normal strain but not with various other viruses. Efforts at changing one strain into the other were unsuccessful. Another, referred to as the changed, strain produced lesions of mixed character, partly inflammatory, partly fibromatous; it continued to behave in this way through numerous passages. An artificial mixture of inflammatory and fibromatous viruses behaved in all respects like the changed strain. Discussion of the significance of the findings is reserved for a separate paper (3) where the facts can be considered in relation to those described by Shope (2).

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EXPLANATION OF PLATE 14

Fig. 1. Section of testis of rabbit inoculated 7 days previously with OA virus. Early proliferation of fibroblasts in the interstitial tissues is shown. × 96.

Fig. 2. Section of testis of rabbit inoculated 7 days previously with IA virus. Intense infiltration with lymphocytic and mononuclear cells is evident. × 96.

Fig. 3. Section of testis of rabbit inoculated 15 days previously with changed strain of virus. The lower part of the picture shows the fibromatous (OA) type of response, the upper and right hand part the inflammatory (IA) reaction. × 96.
Andrewes: Change in rabbit fibroma virus I