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Ticks as Virus Vectors in Eastern Europe

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During the past 20 years there has been a considerable increase in our knowledge concerning viruses transmitted in nature to man and domestic animals by means of arthropod vectors. The present number of these arthropod-borne (arbor) viruses amounts to about 150, of which about 120 are partially classified. Every year new viruses are being isolated either from vectors or wild living animals or man.

The new virus isolations are a result not only of improved virus isolation techniques, but also of changed opinions concerning the role of both the virus circulating in nature and its hosts, especially of its vectors. The starting positions change. Whereas at the beginning there was basically only one problem, namely the sick person or animal and ways how to prevent their infection by known prophylactic measures (vaccination, seroprophylaxis), attention is being paid recently to all factors involved in the maintenance and circulation of virus in nature.

A theoretical basis for such a conception was offered by E.N. Pavlovsky (1939, 1940) in his teaching of natural foci of infection and landscape epidemiology. Pavlovsky (1956) defines a natural focus of infection "as a tract of country of a definite geographical type, containing habitats[x]) in which certain given interspecific relationships have evolved between the pathogen (the microorganism) and the vector or vectors. The latter [illegible] the infectious agent

x) Habitat is a geographically well-defined area characterised by the presence of a particular flora and fauna.

from animal donor to animal recipient under conditions of the external environment conducive to, or preventing circulation of the microorganism among the participants in such biocenose".[xx)] In

[xx)] Biocenose is used here to refer to the "species-network" which is the mutually connected assembly of living species within the ecosystem, the latter being defined as a particular geographical and climatic area together with the whole community of living organisms, both plant and animal, existing in this physical environment.

this definition the term microorganism includes not only viruses, but also bacteria and parasitic protozoa. Emphasis is laid on the ecological relationships between the microorganism, its hosts and the general character of the area, in which the microorganisms pathogenic for man are circulating. This gives rise to the idea that an efficient prevention of transmissible diseases, this being synonymous with arbor virus infections transmitted to the host by vector, will consist of efficient measures taken directly in the field after ascertaining all factors determining the ecology of a virus or of the agent pathogenic for man or animals. In this connection we should like to note briefly that E.N. Pavlovsky agrees with extending his conception on natural foci of infection also to plant viruses transmitted by leafhoppers from wild to crop plants (Valenta 1956).

In mosquito-borne virus infections of man some findings on the ecology of viruses and their hosts were ~~started to be~~ introduced into practise very early. An example is the prevention and eradication of yellow fever in some areas. But even in the case of this classical example of an arbor virus infection so thoroughly investigated, further studies are necessary on virus ecology. The same applies with the huge work done by different investigators, especially by the group of workers of the Rockefeller Foundation in different parts of the world. They isolated many new virus species, many of which (cannot yet be given in a causal connection) [have not yet been linked] with diseases of man or domestic animals and which seem to await in their evolution and ecology their parasitic stage.

In Europe, ticks are known as vectors of viruses of the Russian tick-borne encephalitis complex and of the virus of Crimean hemorrhagic fever. The Russian tick-borne encephalitis complex includes viruses of louping-ill distributed in northern parts of Great Britain and in Finnland (Oker-Blom 1956), of Central European tick-borne encephalitis, of bi-phasic meningoencephalitis (Smorodintsev et al. 1954) and of Russian spring-summer (tayga) encephalitis (Zilber 1939).

The viruses mentioned form a group of antigenically very closely related viruses, the differentiation of which to the individual types, in general corresponding to the slight differences in pathogenesis in monkeys (Ilyenko and Pokrovskaya 1960) and to the clinical picture, was successfully solved by Clarke (1960) by absorption of sera and immunoprecipitation in agar.x Because all the viruses of the tick-borne encephalitis complex mentioned were found in the eastern parts of Europe, they and their vectors will be dealt with individually.

x Viruses from Central Europe were found antigenically identical with the virus of biphasic meningoencephalitis

I. Louping-ill

The only vector of louping-ill virus is the tick *Ixodes ricinus*. The role of this tick in transmission of the infection has become known long before the virus was discovered (Stockmann 1916, 1918, 1919, 1925). Works after 1930 (Pool et al. 1930, Pool 1931 a, b, Greig et al. 1931, Gordon et al. 1932, Mac Leod 1932, Mac Leod and Gordon 1932, 1933, Mac Leod 1936) showed definitely that individual instars of *I. ricinus* ticks collected in pastures where the disease had occurred and those which moulted from infectious preceding instars could transmit the virus to healthy susceptible sheep. The existence of virus has been confirmed in the laboratory. MacLeod and Gordon (1932) did not succeed in demonstrating transovarial transmission, although transstadial transmission was demonstrated on several occasions by the authors mentioned. Transstadial transmission of virus from larval to nymphal instars was shown experimentally in Rhipicephalus appendiculatus ticks by Alexander and Neitz (1933).

I. ricinus females which sucked on viraemic mice, were removed from them and allowed to engorge on susceptible sheep, transmitted the virus to the latter (Grešíková and Řeháček 1959). In the case of a strain of low virulence both the viremia in sheep and excretion of the virus in the milk was erratic and irregular. However, a virulent strain led regularly to a viremia lasting for longer periods and to virus excretion in the milk (Grešíková et al. 1960).

II. Tick-borne encephalitides

1. Isolation of virus from the field

We shall review briefly the history of isolations of the tick-borne encephalitis viruses, i.e. of the Russian spring-summer (the so-called tayga) encephalitis, bi-phasic meningoencephalitis and Central European encephalitis viruses.

In 1938, Levkovich and co-workers (Zilber 1939) isolated a virus causing severe encephalitis in man in the Far East of the Soviet Union. Chumakov and Zeytlyonok (1939) obtained virus strains from Ixodes persulcatus ticks in the Ural and Transural regions by allowing larvae to feed on mice as well as by inoculating mice with tick suspensions. At about the same time the virus was isolated from I. persulcatus ticks by Shubladze and Serdyukova (1939) and Smorodintsev (1939). Pavlovsky and Solovyov (1941) tested experimentally and extended previous findings on the virus circulation in Haemaphysalis concinna ticks (Ryzhov and Skrynnik 1941).

In Central Europe, isolation of tick-borne encephalitis virus from I. ricinus ticks was first reported by Rampas and Gallia (1949) in Czechoslovakia. In Poland, the virus was isolated from the same tick species by Przesmycki et al. (1954), in Hungary by Fornosi and Molnár (1954), in Austria by Verlinde et al. (1955), Grinschgl (1955) and van Tongeren (1955), in Sweden by von Zeipel (1958, 1959) and in Germany by Wigand (1960). Viruses were isolated from man in Yugoslavia (Vesenjak et al. 1955, Kmet et al. 1955, Bedjanič et al. 1955) and Rumania (Draganescu 1959). The occurrence of tick-borne encephalitis in Bulgaria was confirmed serologically (Vapzarov et al. 1954, 1956).

A further species of tick, Dermacentor silvarum, was reported as occasional vector of the spring-summer encephalitis virus. In western Siberia the virus was also isolated from the tick Ixodes plumbeus Leach parasitizing on birds. In Central Europe, the virus was isolated from Dermacentor marginatus ticks collected in nature (Libíková and Mačička 1955, Libíková and Albrecht 1959).

The bi-phasic meningoencephalitis virus was isolated in the Leningrad region from I. ricinus ticks (Smorodintsev et al. 1953).

The percentage of virus infected ticks in nature varies. Smorodintsev (1939) reported 2.5-5% in one locality, but on other occasions 8% (Smorodintsev 1953) or even 25% (Smorodintsev, 1958). In Czechoslovakia there are also differences in different regions in the same year and season, but isolations are on the whole rare. For example Libíková (1956) did not obtain any virus strain from 21,130 I. ricinus ticks, whereas Radvan et al. (1956) obtained 10 strains from 4,680 ticks in another natural focus.

2. The persistence of virus in ticks

The tick-borne encephalitis virus persists in individual instars of engorged ticks during the whole stage until metamorphosis is completed, even if this lasts (e.g. in winter) several months.

in species hitherto experimentally examined, the virus persists in starving ticks after completed metamorphosis during the whole period of the next instar, the duration of which period is variable and depends on temperature and on the time the tick needs for finding a new host. Under experimental conditions the virus persisted in starving I. ricinus females for up to 9 months at both room temperature and +4° C (Benda 1958 a). Under such conditions the virus persisted for varying periods in different tick species: for 28 days in Dermacentor silvarum (Skrynnik and Ryzhov 1940), for 60 days in an Ixodes persulcatus female and 14 days in Haemaphysalis concinna (Smorodintsev 1939) and for 5 days in Ornithodorus moubata (Kolman 1955). The time for which the virus persists in ticks becomes longer if the time of transmission from one instar to another is included: up to 14 months in D. silvarum (Skrynnik and Ryzhov 1940), up to 26 months in I. persulcatus, 12 months in both I. ricinus (Chumakov 1944) and H. concinna (Kozlova et al. 1941).

The percentage of ticks which can be infected experimentally varies from 60 to 90% (Benda 1958 a).

In engorged ticks there was evidence of virus multiplication. In I. persulcatus ticks virus multiplication proceeded up to the 40th day after engorgement; up to the 60th day the virus titer decreased (Shubladze and Serdyuk 1939). Virus multiplication takes place in any instar of I. ricinus ticks after sucking infectious blood. In the course of the first 2-4 weeks the virus titer increases by about 1-3 log units, which is explained by virus penetration into cells of organs and its multiplication therein. After that the titre of virus decreases by about 1-2 log units or it remains unchanged until metamorphosis of the tick (Benda 1958 a).

Graph ~~Fig.~~ 2

When following the titer of virus during the subsequent instars of the tick, a further decrease can be observed (Table 1).

A decisive effect on the amount of virus present in a starving instar of tick and on ~~that~~ whether the virus will persist in the next instar at all is exerted by the host itself, ~~which~~ can be non immune, immune or ~~which~~ can undergo viraemia. In the latter case reinfection of the tick will take place. Blood from a non immune animal will cause either no change in the virus titer in the tick, or its decrease, or ~~these~~ it will be impossible to demonstrate the virus. Sucking the blood on an immune host leads to a decrease in the titer of virus, or to irregular demonstration of virus, or complete neutralization of virus in the tick (Benda 1958 a).

Graph ~~Fig.~~ 3

Antibodies in the host's blood thus exert a considerable effect on the amount of virus present in ticks and, finally, on the amount of virus present during a certain period in the natural focus of infection. Viremia will not develop in immune animals when infested with infectious ticks. Thus immune animals cannot become sources of infection for non infectious ticks, but by their antibodies they can influence considerably the virus in the vectors. The virus titre in the latter can decrease or the virus can be completely neutralized. The latter case can occur only if the virus is present in the intestinal tract. However, if it already spread to the individual tick organs, immune blood can neutralize only the virus present in the intestinal tract. But even in this case the quantitative relations are considerably affected, because the intestinal tract harbors the maximum amount of virus. The next instar contains then ~~ix~~ less virus than if a corresponding instar would have sucked blood from a non immune host.

These findings are of importance in working up tick suspensions for virus isolation experiments. When ticks engorged with immune blood are used, the latter can neutralize virus from infectious ticks in the suspension. ~~(Wattowich~~

of the virus in engorged ticks. They dissected I. persulcatus adults engorged on infectious animals 1, 10, 20 and 25 days after infectious feeding. They found the virus in high titers (10^6) in the intestinal tract for the whole observation period and in titers lower by about one log unit in the salivary glands and ovaries. Small amounts of virus (not titrated) were found in the Malpighian glands, brains and gland. No virus was found in the fat body and chitine. The amount of virus in the ticks was on the 25th day the same as on the 1st day after engorgement. In this experiment the ticks were infected with large amounts of virus so that the dynamics of virus spread into the individual organs could not be demonstrated. In H. concinna ticks the virus was present also in the intestinal tract and salivary glands, the latter having the function of a kind of virus collector.

In the course of feeding on the host the ticks excrete considerable amounts of feces, which are infectious. The feces represent basically dried blood from the host, ~~passaged~~ passed through the intestinal tract of ticks. After engorgement, the ticks excrete also guanine, from which, however, virus was not yet isolated.

When infectious larval and nymphal instars of I. ricinus ticks sucked blood from immune or non immune hosts, virus was present in their feces in about the same amounts. In infectious females, which fed on immune animals, the feces contained considerably less virus than was the case with females feeding on non immune animals, or no virus at all (Benda 1958 b).

The regularity with which the virus occurs in feces of ticks, when the latter are infected with virus, led Řeháček (unpublished data) to check the infectivity of I. ricinus females by examining their feces for the presence of virus.

These findings are also of epidemiological importance. It is reasonable to assume that man can be infected by inhaling infectious tick feces, e.g. when working with sheep wool or furs from animals contaminated with infectious tick feces, or by inhalation in a closed space (Blaškovič 1960). It is not excluded either that inhalation or ingestion of infectious feces can produce infections of host animals (young) in their nests.

Evidence that the tick-borne encephalitis virus multiplies in tick cells and that it persists in them for long periods of time in an active state led to the aim at culturing tick organs and cells *in vitro*. In the Institute of Virology of the Czechoslovak Academy of Sciences in Bratislava two cultivation methods have been developed.

The first represents culturing of surviving tissues *in vitro*. In this case the dissected organs survive in the nutrient medium for about 10-20 days, but there is no cell proliferation. For this whole period clear organ contractions could be observed with the Malpighian gland and sometimes also with the ovaries.

So far, two problems have been investigated with surviving tick tissues. It was possible to infect them with ultrafiltrates of yolk sac cultures of *Coxiella burneti* and to obtain in them after 14-16 days morphologically typical Coxiellae. *Ixodes ricinus* and *Dermacentor marginatus* ticks were used. The development of morphologically typical Coxiellae from the filtrates was delayed as compared with the more rapid development in whole ticks (Kordová and Řeháček 1959).

We also tried to propagate arbor viruses in surviving tick tissues, namely in the fat body and hypodermis, ovaries, Malpighian and salivary glands. Tick-borne encephalitis and louping-ill virus strains were used. Newcastle disease virus served as control. There was no difference in the virus survival in the nutrient medium with or without the tick tissues. However, when using the eastern equine encephalomyelitis (EEE) virus, it survived for considerably longer periods in media with than without the tick tissues. The survival of virus was the longest in the presence of tissues of the fat body and hypodermis. In this case clear-cut multiplication of virus was observed upon inoculating the surviving tissue cultures with small inocula (Řeháček and Pešek 1960, Řeháček 1960).

Graph ~~Fig.~~ 4

~~A~~ Much more difficult, but more important is the cultivation of proliferating tick cells. By chromatographic analysis it was found that tick hemolymph contains large amounts of glycides. We therefore enriched the usual nutrient medium with dextrane. Explants from developing Dermacentor marginatus or D. pictus adults are placed in this medium. A two to threefold halo around the explant can be obtained, some cultures survive for more than 20 days. However, the growing of larger amounts of cells and their subcultures were as yet unsuccessful (Řeháček and Hána 1961).

Fig. 1, 2, 3

Although the work with tick tissue cultures for the propagation of arbor viruses was not yet very successful, we will continue our efforts, because they could contribute to the elucidation of several theoretical and practical problems involving the biological properties of the viruses under consideration, their diagnosis and epidemiology.

3. Transstadial and transovarial transmission of the tick-borne encephalitis virus in ticks

There has been usually no difficulty in any laboratory to demonstrate transstadial transmission of the tick-borne encephalitis virus in ticks, which represent its natural hosts. Any tick instar can be infected with the virus and the latter can be recovered from a suspension or by allowing to feed the next starving instar on a susceptible host. In this way it was possible to transmit the virus ~~from one to the next instar~~ through all instars of a generation of both D. silvarum (Ryzhov and

Skrynnik 1939, Skrynnik and Ryzhov 1941) and I. ricinus (Benda 1958 of ticks, through three generations of I. persulcatus ticks (Chumakov 1943) and from larval through nymphal instars to adults of I. hexagonus (Streissle 1960).

The amount of virus in the subsequent instars of ticks decreases in the course of transstadial transmission, depending on the immunity or susceptibility of the host animal. When the tick is feeding on an immune host, this decrease in the virus content in the next instar is more pronounced (Benda 1958 a).

An interesting problem is the transovarial transmission of the tick-borne encephalitis virus in ticks. The results reported are not unequivocal. In interpreting them two circumstances must be taken into account: virus in nature, the natural hosts of which are certain wild animals and a certain species of ticks on the one hand, and virus passaged for long periods of time in laboratory animals on the other. Only experiments done with ticks and viruses originating from the same locality can be compared.

Transovarial transmission was demonstrated in D. silvarum (Skrynnik and Ryzhov 1941), H. concinna (Ryzhov and Skrynnik 1939), I. persulcatus (Serdyukova and Shubladze 1941, Shubladze 1944, Dumina 1958), I. ricinus (Chumakov 1944, Benda 1958 a) and I. hexagonus (Streissle 1960) ticks.

A regular transovarial transmission was reported especially in the first studies on the role of ticks as vectors of tick-borne encephalitis virus. Recently, however, transovarial transmission in ticks from new localities is considered to be irregular (Dumina 1958, Benda 1958 a). Benda (1958 a) estimates the transovarial transmission at 6% and is of the opinion that under the conditions prevailing in Czechoslovakia the transovarial transmission of the virus in I. ricinus ticks is infrequent in nature. Streissle (1960) obtained in I. hexagonus ticks a transovarial transmission rate of

18%. There are several authors who could not demonstrate in their experiments a transovarial transmission in different tick species (van Tongeren 1957, Grešíková and Řeháček, unpublished data). It seems that these differences are not the result of different methods used, but that they are a consequence of the conditions discussed above. According to Smorodintsev (1958) ~~(personal communication)~~, in some foci in the U.S.S.R. ~~more than~~ 25% of ticks are vectors and reservoirs of virus. It would seem that the virus is more adapted to tick organs under these conditions than under the conditions of Central Europe, where the natural foci of infection are not so valid.

4. Transmission of the tick-borne encephalitis virus by ticks to host animals

Transmission of the virus by ticks to host animals or man takes place either biologically (by bite of a starving tick) or mechanically by the so-called interrupted feeding.

Biological transmission is the normal way of transmitting the virus by means of ticks. In this way the virus can be transmitted to host animals by any of the tick instars. According to Petrishcheva and Levkovich (1949) a two-day-feeding of three infectious larvae of I. ricinus or I. persulcatus is sufficient to cause encephalitis in mice. One infectious I. ricinus female is sufficient to cause fatal infection of a mouse (Benda 1958 b). The time for which the ticks can transmit the virus is very long. In I. persulcatus it is 26 months and the virus passes through 3 generations (Chumakov 1944). The greatest amounts of tick-borne encephalitis virus are excreted by salivary glands of females, smaller amounts by those of nymphs and the smallest by those of larvae (Benda 1958 b). This is in contrast with the experiences of Soviet investigators (Smorodintsev, personal communication), who recommend to collect engorged females in the field and to use larvae hatched from them for virus isolation experiments.

After a bite by an infectious tick viremia of varying duration develops in susceptible hosts (the duration presumably depends on the degree of susceptibility to infection). Viremia does not occur in animals which acquired immunity following a previous contact with virus. In their blood haemagglutination-inhibition and virus neutralizing antibodies can be detected. After a fresh infection with even small doses of virus, the host animal produces also complement fixing antibodies in addition to virus neutralizing antibodies.

It is reasonable to assume that also in nature virus transmission to an animal host can occur by an interrupted feeding. When catching ticks, also partially engorged individuals are caught. Under experimental conditions, virus transmission by interrupted feeding was demonstrated in I. persulcatus males and females, in the case of the females after a 4-hour-feeding on sick mice or men, from whom the ticks were transferred after 3, 6, 7 or 8 days on healthy mice, on which they fed until completely engorged.(Chumakov 1939, Shubladze and Serdyukova 1938). Virus transmission by interrupted feeding was also demonstrated in D. silvarum (Skrynnik and Ryzhov 1940). The Central European strain of tick-borne encephalitis virus could also be transmitted by interrupted feeding of I. ricinus females to goats (Grešíková and Řeháček 1959).

5. Hibernation of tick-borne encephalitis virus in ticks

One of the main problems of the biology of tick-borne encephalitis virus in nature is the persistence of virus during interepidemic periods or during those in which the ticks are inactive, i.e. in winter. Levkovich and Skrynnik (1940) collected different tick instars in the spring and tested them for presence of virus. Thus they succeeded in obtaining several strains of the tick-borne encephalitis virus in the Khabarovsk region. Experimental studies on transovarial transmission or on the persistence of virus for varying periods of time and at different, including low, temperatures offer unequivocal evidence of that the tick-borne encephalitis virus hibernates in ticks. In our Institute, Řeháček (1960) observed virus survival in engorged I. ricinus larvae for 102 days of hibernation. He could recover the virus from the larvae on the 6th and from the hatched nymphs from the 57th - 88th days after the end of hibernation. Loew (1960) observed virus survival in ticks after 8 months of winter rest in terraria with optimal biological conditions.

III. Crimean hemorrhagic fever

Crimean hemorrhagic fever occurs in the Crimea and was also found in Bulgaria and Central Asian Soviet Republics. The virus was isolated from filtrates of Hyaloma plumbeum plumbeum ticks from the field. Transmission to man takes place only by tick bite. However, infections were reported of nurses who came into direct contact with patients' blood. In 1944 in the Crimea tick bite was reported in the anamnesis of 87.8% of patients and in the rest it was not excluded. The distribution area of the disease corresponds with that of H. plumbeum plumbeum and H. anatolicum ticks. H. plumbeum larvae and nymphs were found the most often in hares, but no virus could be isolated from their blood.

The period of activity of H. plumbeum ticks begins in April, reaches a maximum in July and August and drops in September. The ticks are living in the steppe on the ground, grasses and shrubs. Both adults and nymphs of the ticks can feed on man. Transovarial transmission was demonstrated in H. plumbeum ticks. The virus can overwinter in the ticks. (The above data are quoted after Chumakov 1957 and Gapochko et al. 1957).

We have presented some data on the relationship of viruses circulating in nature to ticks acting as their vectors or, eventually, as their reservoirs. We are aware that we could not exhaust all the problems in their whole width; we only pointed out some of them, in which we are interested the most. We think that the data reported will help to elucidate the ecology of tick-borne viruses in nature with the aim to interrupt the circulationx of these agents in order to prevent the danger of infection of man and of economically important animals.

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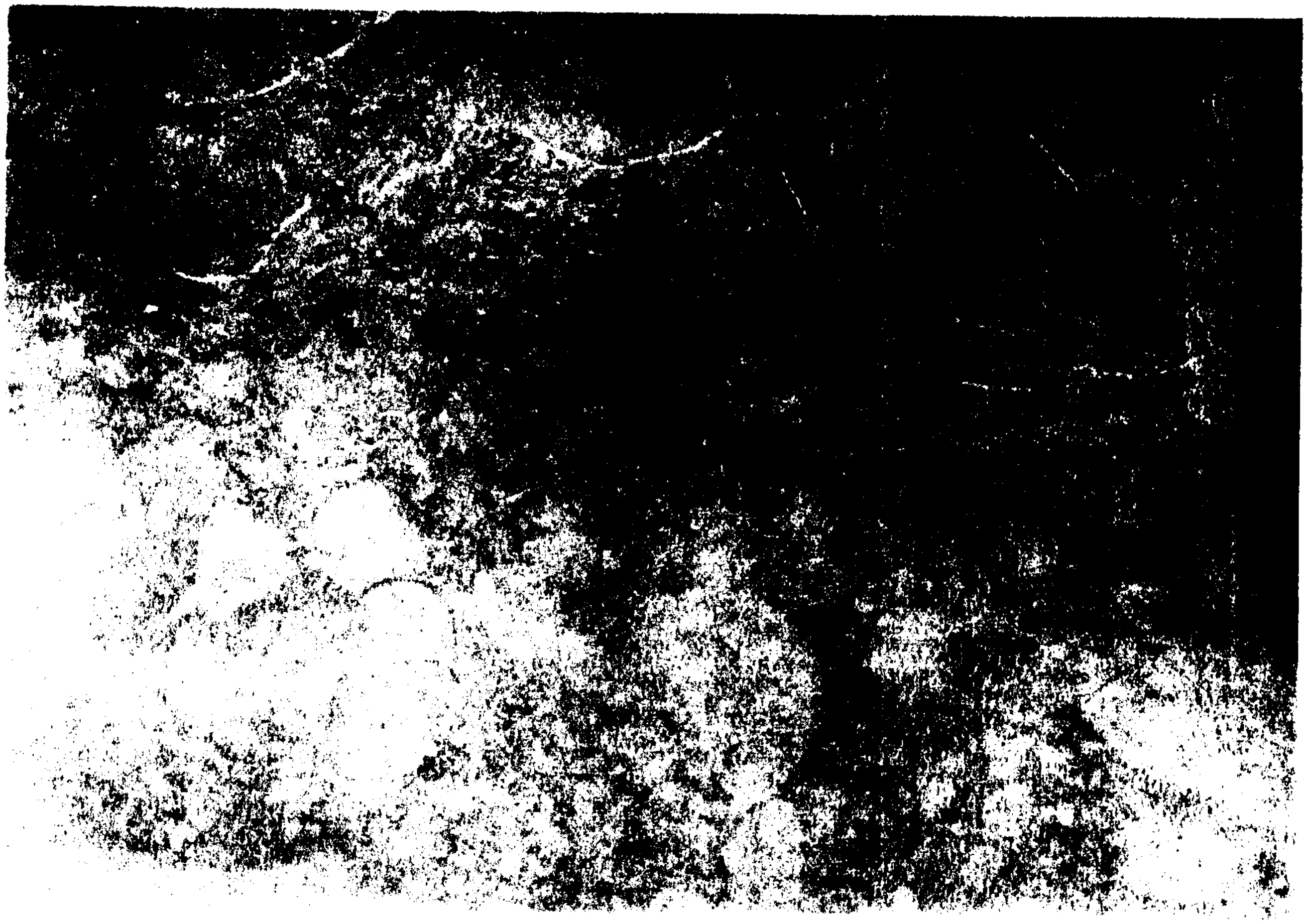

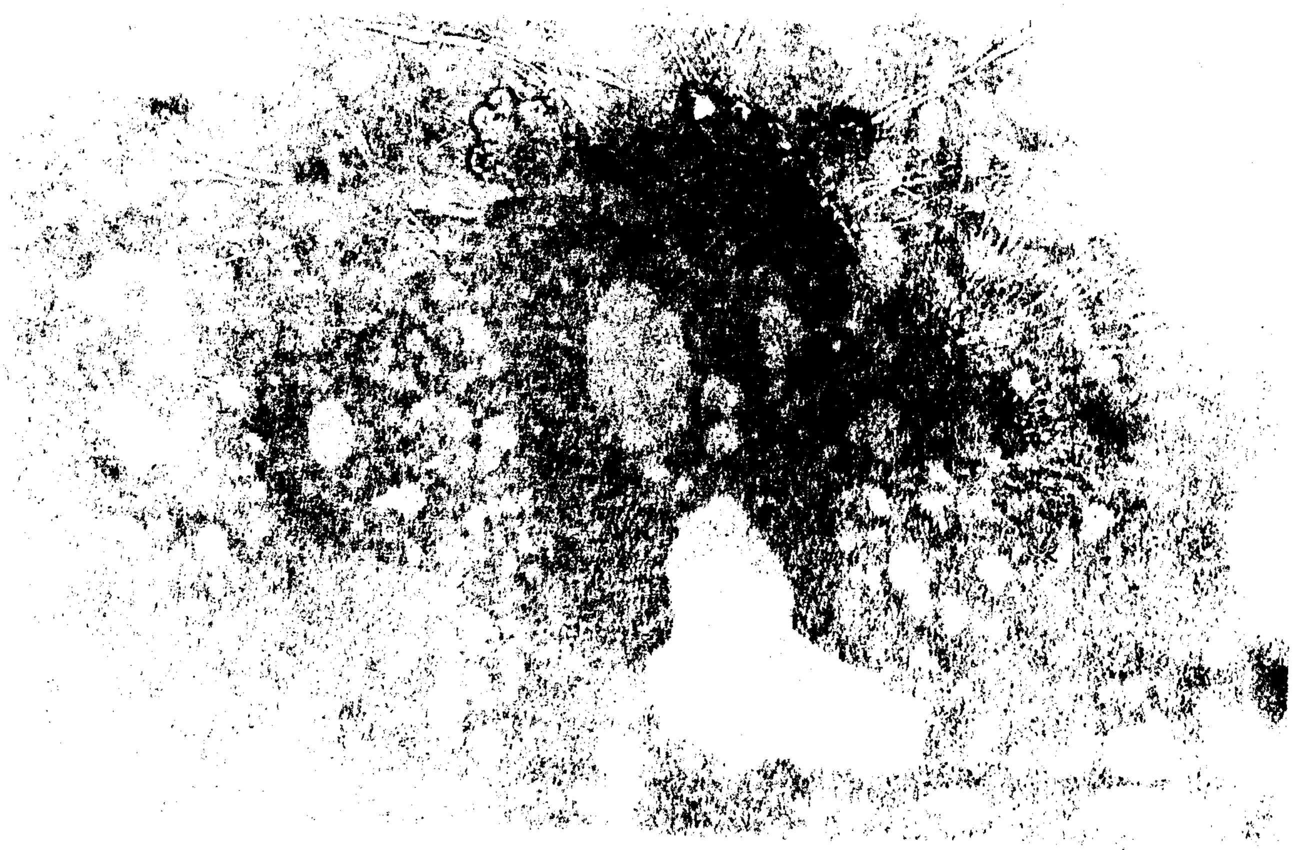

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