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An Epidemiological and Hematological Study of Sandfly Fever in Serbia.

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Sandfly fever in Yugoslavia was well known in the areas under the direct influence of mediterranean climate: Dalmatia, Montenegro, Herzegovina and Macedonia. However, in the region of Serbia it was unknown until 1946 when it broke out and assumed the intensity of an epidemic, spreading over the whole country. Since that time sandfly fever occurs every year in smaller or greater epidemics. The northern part of Serbia—Vojvodina district—where the epidemics broke out as late as 1948, was specially affected with great severity and approximately three-fourths of its population (1,200,000) went down with sandfly fever.

The conditions for development of phlebotomae were unusually favourable during four successive warm and drought years and these insects became annoying molestants to the great surprise of inhabitants to whom they were unknown before. The virus was imported from the southern parts, mainly from Macedonia, where sandfly fever occurs in endemic form. Thus endemic regions were moved up northward for 600 km. towards the Hungarian frontier and apparently passed it, spreading over its southern parts.

In the south and middle of Serbia the most affected communities were those lying in the flats along the big rivers, while in the northern part (Vojvodina district), which is flat land allover, no village was spared.

Regarding Belgrade city, the greatest number of cases occurred in low lying quarters on the banks of Sava and Danube rivers, though the quarters situated higher up were by no means excepted. The infection did not comprise only the people who had their homes on the ground level, but also affected those living in houses with many stories. *P. papatasi* was frequently found in top stories and attics as well.

In habitations affected for the first time with sandfly fever the cases at the beginning of the epidemic were grouped just in one area, thus giving the impression of a contact transmission. This picture of contact spreading is retained only for a short time, about 2 to 3 weeks, and then abruptly the sandfly fever spreads with scattered cases over the other parts. The localisation of the epidemic in its early phase is undoubtedly due to the short flight range of *P. papatasi*.

The seasonal distribution of sandfly fever in Serbia is from the second half of June to the second half of September, namely from the appearance of a greater number of sandflies in living quarters and their noticeable night activity till their marked reduction.

In the communities of Vojvodina district the same persons were affected with sandfly fever two to three times during one year, and likewise there were many cases who had infections during two successive years. In our estimation recurrent cases reached as high as 21%. All recurrent cases occurred during the seasonal activity of *P. papatasi*, and thus it may be that we are dealing with the reinfections rather than with relapses. This theory is strengthened by the fact that in Belgrade city recurrent cases were observed only as exceptions. In the villages of Vojvodina district *P. papatasi* was found in great abundance;

sometimes as many as a hundred or more insects were captured just in one room, whilst in Belgrade city with its many storied houses their dispersion was greater and thus the possibility for reinfections decreased. Most of the captured insects in patients' rooms were of the *P. papatasi* species. Only in a few instances other species like *P. perfiliewi* and *P. major* were found. Out of 500 examined insects, 85% belonged to *P. papatasi*, 13% to *P. perfiliewi* and 2% to *P. major*. The ratio between species varied according to the locality. The determination of species was carried out by the entomologist V. Živković.

For clinical investigations we chose a rural community in Vojvodina district where sandfly fever cases occurred for the first time in 1951. The total number of people we examined was 80. Males and females were attacked in the same proportion: males 39 and females 41. As to the age distribution it seems that all age groups are equally susceptible to sandfly fever infections, with the exception of early ages up to 5 years. Our youngest patient was 4 years old. The smallest number of cases in the age groups 60-80 years does not necessarily mean decreased disposition of old people to this disease, but the reason lies in the fact that in rural communities there are few old people. Contrary to this, we found that in children up to 5 years there exists a resistance to sandfly fever which is expressed in symptomless infections. Also the clinical course among children up to 10 years was markedly mitigated. Hereditary immunity cannot be taken into consideration, since Vojvodina district became acquainted with this disease for the first time during the same year.

TABLE 1.
Sandfly fever by age groups.

| Years | 1-10 | 11-20 | 21-30 | 31-40 | 41-50 | 51-60 | 61-70 | 71-80 |
|--------------|------|-------|-------|-------|-------|-------|-------|-------|
| No. of cases | 5 | 15 | 12 | 11 | 15 | 13 | 5 | 4 |

The incubation period limits could not be fixed during the course of epidemics. We are not going to dwell on clinical symptoms, since their classical description is already given in most textbooks. However, we shall mention that gastro-intestinal disturbances and bleedings from mucous membranes (epistaxis) were more frequent during epidemics in 1952 than in 1951. No rash was observed during any of these epidemics. Complete loss of appetite and bitter taste in the mouth was almost a regularity among our patients. Many of them, especially among old people, endured their illness without taking even a drop of liquid.

Hematological investigations. Hematological investigations were carried out on 77 patients during the febrile stage of the disease and in convalescence.

It is characteristic of sandfly fever that the changes occurring in the number and differential count of white cells at the onset of the disease remained nearly the same throughout the febrile period and the first week of convalescence. The basic feature of hemogram in sandfly fever is leucopenia. The diminution in the white cell count is rather pronounced and amounts on average to 4,300 cells per cmm. Generally in virus diseases there is leucopenia with relative lymphocytosis. However, *Sabin, Philip and Paul* (1944) describe in experimental sandfly fever a leucopenia due to the fall in the lymphocyte count. In the cases of sandfly fever we have been investigating lymphocytes were found in 44.1% of all white cells, or in absolute number 1900, which is above their lower normal limit. In the epidemics of sandfly fever in Serbia, contrary to the findings of the above-mentioned authors, a neutropenia with relative lymphocytosis was marked, as is the case in most virus diseases.

The neutropenia was accompanied by a great increase in stab cells. The stab cells in relation to lobed granulocytes during the onset and first three days of convalescence amounted to 42.3%. Further in the course of convalescence the number diminished, amounting to 24% on the 9th day. Notwithstanding the great percentage of stabs the shifting to the left is arrested at metamyelocytes. In the cases of sandfly fever we investigated there were very few metamyelocytes, while more primitive forms of these white cells do not come at all in the blood picture of sandfly fever. The nuclei of stab cells show in their structure condensation of chromatin, and judging by it these forms might represent the final stage of maturation. In certain severe toxæmia, according to *Schilling*, the reaction of white-cell formation centers of bone marrow shows a degenerative character, expressed in leucopenia and a large number of stab cells which have failed to segment.

As we have already seen, lymphocytes were not diminished, being generally in absolute number above their lower normal limit. In nearly all cases of sandfly fever small lymphocytes were represented by a low percentage, while large ones dominated the white blood picture. The ratio between small and large lymphocytes in sandfly fever was changed in inverse proportion: 56.5% large and 43.5% small lymphocytes. It was not uncommon that nuclei of large lymphocytes contained nucleoli or their relics, while in cytoplasm there were coarse azurophil granules, a sign of dissociation of nuclear and cytoplasmic maturation.

A fairly common appearance in the white blood picture were the granulocytes with pathological granulation and vacuolation. Further, smear cells, basket cells and senile cells amounted to 14% of all white cells. All these morphological alterations in white cells bore a degenerative character and point to a severe toxæmia developed in the course of sandfly fever.

In the patients we investigated the number of monocytes was not increased, neither in the febrile nor in the postfebrile period, but some slight increase in monocyte count was apparent from the 8th day of the beginning of the disease.

TABLE 2.

Total and differential count of leucocytes in sandfly fever from the onset till the 9th day of disease.

| No. of day from the onset | Leucocytes per 1 cmm. | Neutrophil metamyelocytes in % | Neutrophil stab forms in % | Neutrophil segmented polymorphs in % | Eosinophil polymorphs in % | Basophil polymorphs in % | Lymphocytes in % | Monocytes in % |
|---------------------------|-----------------------|--------------------------------|----------------------------|--------------------------------------|----------------------------|--------------------------|------------------|----------------|
| 1 | 4375 | 1.0 | 29.0 | 38.0 | 1.0 | 0.5 | 26.5 | 4.0 |
| 2 | 4300 | 0.5 | 24.0 | 24.2 | 1.0 | 0.2 | 46.0 | 4.0 |
| 3 | 4125 | 0.5 | 22.0 | 23.0 | 1.3 | 0.5 | 49.0 | 3.7 |
| 4 | 3400 | 0.5 | 20.0 | 21.0 | 2.0 | 0.5 | 52.0 | 4.0 |
| 5 | 3500 | 0.5 | 22.0 | 18.0 | 2.0 | 0.5 | 52.0 | 5.0 |
| 6 | 4000 | 0.5 | 17.0 | 22.0 | 1.0 | 0.5 | 56.0 | 3.0 |
| 7 | 4400 | 0.0 | 31.0 | 41.0 | 0.0 | 0.0 | 26.0 | 2.0 |
| 8 | 5400 | 0.0 | 11.0 | 34.0 | 1.0 | 0.5 | 47.0 | 6.0 |
| 9 | 5000 | 2.0 | 10.0 | 32.0 | 2.0 | 0.0 | 43.0 | 10.0 |

The number of blood platelets in sandfly fever is often reduced, amounting on average to 200,000 per cmm. Furthermore the platelets were often markedly increased in size, sometimes reaching that of a red cell, 6-8 μ in diameter. The shape of these giant platelets is round, oval, elongated or irregular. They were often found in agglomerations, but also separately scattered all over the blood films. The giant platelets were considerably less granulated than platelets of normal size. Platelets of a larger size are not a rare occurrence in certain infectious diseases or in blood disorders accompanied by thrombocytopenia, especially in Werlhof's disease. These large forms of platelets are a fairly common occurrence in the blood films taken from the patients of sandfly fever. Obviously the thrombocytopenia and giant platelets are to be considered as a sequel to a severe intoxication of bone marrow. The peripheral destruction of blood platelets as a cause of thrombocytopenia does not come into consideration, for in sandfly fever there is no splenic enlargement, and so the giant forms of platelets speak against it. But whether these giant forms of platelets are to be considered as a compensatory reaction of bone marrow in thrombocytopenia, or merely as a degenerative reaction, remains an open question. It is worth mentioning that in our cases of sandfly fever, though the platelets were considerably diminished in number, there were no purpuric spots in the skin and very rarely hemorrhages from the mucous membranes.

All the above described changes in granulocytes, lymphocytes and blood platelets: neutropenia, increased number of stab cells with arrest of the shifting to the left by metamyelocytes, the maturity of nuclei of stab cells, a great number of senile cells and cells with toxic granulation, inverse ratio of small and large lymphocytes and finally thrombocytopenia with giant platelets point to a particular mode of reaction on the part of bone marrow and lymphoid centers to the toxins created in the course of the disease by virus invaded cells. The part played by these toxins becomes evident in the altered reaction of three hemopoietic centers: myeloid, lymphoid and megacaryocytes. With regard to these reactions, they are probably of a degenerative character, for not only the changes in peripheral blood speak for it, but also its long persistence throughout convalescence. With neutrophil granulocytes the return to normal occurs earlier than it does with lymphocytes. It is for this reason that we believe the centers of lymphopoiesis are more affected by toxins and their reactions are more profoundly altered.

The centers of erythropoiesis are not affected by toxins created in the course of sandfly fever, nor are the red blood cells in peripheral circulation altered. Some increase in the counts of red blood cells was found in a certain number of our patients, amounting on average to 300-500,000 cells per cmm. This transitional increase in the count of red blood cells was due to the blood inspissation. The cause of blood inspissation lies in the patient's refusal to partake of any food and even liquids, which is a regular occurrence in sandfly fever, further in sweating and finally in diarrhea and vomiting in some cases. The highest erythrocyte count (6,800,000 per cmm.) was found in one old patient who, during the febrile period and two days of convalescence, did not take a drop of liquid.

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