regarded as the analog of the bursa of Fabricius, but justifies the conclusion that it is a peripheral organ participating in local immune reactions and forming part of the general system of immunity.

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A NEW HEMATOLOGIC METHOD OF DETECTING LATE SEQUELAE OF TRANSIENT MYOCARDIAL ISCHEMIA

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Changes in the cell composition of the peripheral blood in experiments on animals have been studied only during the first few hours after "subcritical" [11], transient myocardial ischemia [10]. Routine hematologic tests in the subsequent period can no longer reliably reveal evidence of previous transient disturbance of the blood supply to the heart [1]. Moreover, as has been pointed out, even in the case of irreversible disorders of the coronary circulation, involving the development of a myocardial infarct, the cell composition of the peripheral blood showed changes during the next few days in only a proportion of cases [9].

Accordingly the writers suggest a new technique for hematologic monitoring of the sequelae of reversible disturbances of the coronary blood flow, during the use of which repeated determinations of the cell composition of the blood are combined with a function test that stimulates an increase in the number of circulating leukocytes. The object of the present investigation was to test the effectiveness of this method of discovering latent postischemic leukocytosis by the use of a model of reversible local disturbances of the coronary blood flow in chronic experiments on dogs.

EXPERIMENTAL METHOD

To prepare the animal for the experiment a device for controlling the blood flow in the circumflex branch of the left coronary artery was implanted [5]. Some 2 to 3 weeks later the animal was used in the experiment, for which either the animal received no medication or it was preceded by a single intramuscular injection of 0.1 mg fentanyl and 5.0 mg droperidol. These drugs were given only when the dog strapped to the frame exhibited increased motor activity. Altogether 21 chronic experiments were performed on five animals, in which the leu-

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kocyte and erythrocyte counts, leukocyte formula, ESR, hemoglobin concentration, and hematocrit index were determined. In each experiment blood was collected twice from the animal's ear: before application of a rubber tourniquet, interrupting the blood supply for 13-20 min, to 1 limb, then 30 min after removal of the tourniquet. By comparing the results of these two hematologic investigations the response of the blood system to temporary disturbance of the circulation in the limb was determined in each experiment, and this served as a function test stimulating an increase in the number of circulating leukocytes [2]. In the main series of experiments (11 observations) the response of the blood system to the tourniquet test was studied 24-120 h after reversible myocardial ischemia for 3-5 min. The ten control observations were made on the same animals, but during a period of the chronic long-term experiment when disturbances of the blood flow in the circumflex branch of the left coronary artery were not preceded for six days or more by the tourniquet test.

EXPERIMENTAL RESULTS

Background values (before the tourniquet test) of the principal hematologic parameters were as follows (in 1 mm^3 blood): total leukocyte count 9300 ± 700; erythrocyte count 3.9 ± 0.1 million, Hb concentration 14.6 ± 0.3 g%, hematocrit 41.16 ± 0.21%, in agreement with the normal values for animals of this species [4, 7]. Some tendency for the mean values of the erythrocyte count, Hb concentration (by 1.14 g%), and hematocrit (by 1.0%) to increase was observed, accompanied by a decrease in the total leukocyte count (by 100) in the main series compared with the corresponding values in control experiments conducted on the same animals. However, this difference was statistically significant ($P < 0.01$) only for the erythrocyte count: 3.8 ± 0.1 million in the control and 4.2 ± 0.07 million in the main series of experiments.

The tourniquet test in the main series of experiments, conducted 24-120 h after transient myocardial ischemia, caused an increase in the total number of circulating leukocytes on average by 5680 ± 1290 ($P < 0.01$). In the control series the reaction was completely different: a small increase or, more frequently, a decrease in the leukocyte count. The mean leukocyte count was reduced in the control series of experiments by 970 ± 630 as a result of the tourniquet test. The difference between the reactions in the two series of experiments was statistically significant ($P < 0.001$).

If the tourniquet test was carried out during the first 24-48 h after reversible myocardial ischemia, the increase in the total leukocyte count was accompanied by a neutrophilic shift of the leukocyte formula in every case. The number of neutrophils was increased on average under these circumstances by 5.7 ± 2.1% ($P < 0.05$). If the tourniquet test was performed later, 72-120 h after transient myocardial ischemia, a tendency was observed for the lymphocyte or monocyte count to increase more often than the neutrophil count. Besides changes in the leukocyte count after the tourniquet test, changes also were observed in the red blood picture. There was a particularly marked tendency toward a greater decrease in the erythrocyte count in the main series of experiments (by 0.47 ± 0.1 million) compared with the control values (by 0.16 ± 0.09 million). In two dogs, after a series of experiments with reversible disturbances of the coronary blood flow, separate changes in Hb concentration, hematocrit, and erythrocyte count in different experiments also reflected both stimulation and inhibition of erythropoiesis. These same phenomena are observed in this particular experimental model even in the absence of tourniquet tests [10]. Another noteworthy feature was that in one of eleven cases in the main series of experiments the response of the blood system to the tourniquet test was not typical but consisted of a definite decrease in the number of circulating leukocytes. It must be assumed that this variant of the changes was not accidental. The cause of the differences may evidently be that the effect of disturbances of the coronary circulation in each of the experimental animals was not absolutely identical in all experiments. Moreover, inconstancy of manifestation and variability of the leukocyte response are also found under different experimental conditions. such as in a model of alimentary leukocytosis [3, 8].

On the whole the data indicate that under chronic experimental conditions the last sequelae of "reversible" disturbances of the coronary blood flow can be reliably established on the basis of a number of hematologic parameters involving the tourniquet test with temporary interruption of the circulation in the limb. The diagnostic value of this test is evidently attributable to the action of several factors. First, in animals with an intact myocardium, 30 min after the tourniquet test the number of circulating leukocytes may be stabilized at a relatively low level as a result of the development and subsequent normal evolu-

tion of the so-called "leukocyte response to pain." The minimal trauma to the unanesthetized animal required for such a response to appear may be provided by the taking of the first blood sample from the animal's ear and subsequent compression of the limb tissues by the tourniquet. Second, in animals with an intact myocardium the leukocyte response to the tourniquet test [2], like any other similar relatively mild response, may be accompanied by a marked initial leukopenic phase. It is thus quite logical to expect that after "subcritical," "reversible" disturbances of the coronary blood flow both the time of development of the leukocyte response to the tourniquet test and the amplitude of the response itself ought to change significantly, most probably in connection with the previous development of latent postischemic inflammatory changes in the myocardium of our experimental animals and subsequent changes in the threshold of excitability of their cardiovascular center toward afferent influences arising outside the cardiovascular system. It was shown previously [6] that irreversible ischemia of heart muscle may have sequelae detectable by action directed to certain receptor zones of the limb. However, none of the methods used previously allowed such effective and prolonged observation of the sequelae of myocardial ischemia of such short duration (3-5 min) as did the use of the methods described in this paper. Some observations associated with feeding of our animals, as well as others made on other experimental models [3, 8], suggest that not only the tourniquet test, but other function tests may also be used in analogous experiments.

In our opinion, the further development of this method of detecting latent postischemic leukocytosis and various hematologic shifts associated with it must lead to further significant widening of the range of diagnostic procedures for use in various forms of ischemic heart disease.

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