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Biochemical studies on the behavior of the leucocytes after intravenous administration of alkalinized salvarsan.

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I.

The reaction of the involuntary nervous system to intravenous injection of salvarsan, neosalvarsan and silver salvarsan, and the influence of this reaction on the neutrophile leucocytes have been discussed in two previous articles.¹

It was found that in humans the number of white blood cells decreases for a short period immediately following an intravenous injection of salvarsan. This sudden decrease is prolonged in patients who present manifestations of the so-called nitritoid crisis. An intravenous injection of silver salvarsan, however, is not followed by a similar reaction.

These findings, which were discussed at length in the foregoing articles, led to a study of the details of this leucocytic reaction, the main problem being: Where are the leucocytes during the period of their absence from the periphery? It seemed probable that they had migrated to the inner organs. But no proof of this theory could be elicited from the human body, as it is impossible to examine intimately the inner organs of human beings after salvarsan injections except in rare instances, and never in a sufficiently large number of instances to warrant definite conclusions. Furthermore, it is known that abdominal operations, as well as general narcosis, are in themselves productive of a similar decrease in the number of leucocytes in the peripheral vessels. The only avenue of approach to a solution of this problem, therefore, was a study of the conditions resulting in the animal body.

II.

Animal experiments along these lines necessitated preliminary comparative tests. In different series of animal tests a similar decrease in the number of leucocytes was observed after the

¹*Arch. Derm. and Syph.*, 1924, x, 316, 606.

intravenous injection of salvarsan. The decrease is less pronounced or is entirely absent when silver salvarsan is administered.

The object of this series of tests was accomplished by determining the number of white blood cells in the heart blood. The heart blood for the count was obtained by puncturing the heart of the animal. It was observed that there was a practical correspondence in the number of leucocytes ascertained in blood from the peripheral vessels, from the tail vein and from the heart in the majority of animals, both before and after they had received salvarsan injections. From these experiments it was evident that the missing leucocytes were displaced from the peripheral vessels as well as from the heart and the main arteries, but it could not be determined whither they had disappeared during the period of peripheral leucopenia.

Further preliminary tests with normal rats were necessary to determine the leucocytic count in different organs after killing a normal animal. It was observed that immediately after death the blood count, made with heart blood, revealed remarkably lower values than those obtained before death occurred. The number of leucocytes found in the blood of the liver at the same time was very high; for instance 6,000 leucocytes in the heart blood corresponded to 12,000 or more in the liver. Different methods of killing the rats did not succeed in eliminating this factor of leucocyte displacement which, to a certain extent, resembled the change in the leucocyte count after the intravenous injection of salvarsan.

The only possible conclusion was that the act of killing by different methods produces a similar reaction on the leucocytes, and that this reaction in turn produces the displacement from the periphery and the heart to the liver. A continuation of the investigation of this problem along the lines of our former studies was not possible until this reaction could be avoided.

It may be interesting to mention that in rats dying with convulsions, the difference between the cell count in the heart blood and in the blood of the liver was more marked than in those animals in whom few or no convulsions were observed.

III.

When we succeeded in finding a method to kill a rat quickly enough for experimental purposes without producing such sud-

den reaction on the part of the animal as might involve a measurable change in the blood count, the elimination of the above mentioned reaction at the time of death became a reality. It is known that the intravenous injection of air is fatal, due to embolism of the pulmonary arteries. When killing a rat by this method practically no difference is observed between the cell count of the inner organs, especially of the liver and of the heart. The injection of 0.2 to 0.3 cc. of air into the vein of the leg was found sufficient to cause death without any measurable organic reaction. Based upon this knowledge, it was possible to begin a new series of tests to help locate the leucocytes missing after salvarsan injections.

The usual dose of salvarsan—90 to 180 mg. per kilo body weight—was given intravenously in the usual way; 0.3 cc. of air was injected either immediately or 2 minutes after the salvarsan administration. No appreciable difference in the number of white blood cells, both in the heart and in the liver, either before or after injection, was found in the animals injected with air only. The rats, which had received a salvarsan injection before death was induced by embolism, showed the following: There was a marked difference between the first count and the later findings in the blood of the heart and of the liver. The following example will demonstrate:

11:20 o'clock, blood from the tail.....	13600	leucocytes
11:25, salvarsan, 180 mg./kg. body weight, 2 percent solution.		
11:26, blood from the tail.....	9600	“
11:26.40, blood from the heart	10000	“
11:26.50, 0.3 cc. air intravenously.		
11:27, dead.		
11:30, blood from the heart	6800	“
	8400	“
blood from the liver	14200	“
	13000	“

From this and many similar tests it appears that the leucocytes, disappearing for a short period from the peripheral vessels, are retained in the liver for the time being. Our findings do not, however, allow of a conclusion as to whether or not other organs, such as the spleen, participate in this retention of the missing leucocytes, for it is not possible to determine the cell count of the spleen and of other areas of the abdominal circulation with sufficient accuracy to warrant definite conclusions. It is furthermore not always possible to apply the results of animal experimentation to the human economy; but considering the similarity of the

phenomena observed in the human and in the animal, the following can be asseverated.

After salvarsan injections in animals there ensues a peripheral leucopenia of short duration, during which period the leucocytes are mechanically retained in the blood of the liver. A short leucopenia after salvarsan injection is also observed in the human. It is highly probable that the leucopenia in the human observed after injection of salvarsan is also due to a similar retention of the leucocytes in the blood of the liver.

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On the antigens of red blood corpuscles.

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Experiments reported in a previous paper¹ led to the view that specificity as manifested by precipitin reactions on serum proteins and agglutinin or lysin reactions on red cells is based on structures of two different sorts. While the differences are apparent in the case of heterogenetic lysins and isoagglutinins, the findings in agglutinins and lysins in general seem to indicate the existence in the antigens of substances other than proteins (but probably bound to them).

As a matter of fact, the antigenic properties of blood cells have been ascribed to "lipoid" substances by several authors since the work of Bang and Forssman.² It was difficult to accept this notion in view of the fact that extracts of erythrocytes made with organic solvents and supposed to contain the antigen have a feeble action as compared with the original material, and that the residues after such extractions are still active (Thiele and Embleton).

¹ *J. Exp. Med.*, 1924, xl, 91.

² *Beitr. z. chem. Phys. u. Path.*, 1906, viii, 238. The bibliography will be given in the more detailed article on this subject. Cf. Schmidt, H., *Zur Biologie der Lipoid*e, Leipzig, Kabitzch, 1922.