incubation. These fibroblasts from the heart, trunk and intestine of the chick embryos, grown in Tyrode solution, were exposed to the same light source which caused death of blood cells in from 5 to 80 minutes. At the end of 18 hours the fibroblasts were practically normal. On the other hand, cultures from the same groups of slides, inoculated in Tyrode solution plus autogenous washed red blood cells, when exposed to the same light source, showed a striking degeneration in from 1 to 3 hours. In this process of degeneration the cell either became packed with water vacuoles, or else the cell contents showed the same increased fluidity as that shown by the degenerating leucocytes. In both cases the cells gradually rounded, and both nucleus and cytoplasm became edematous. As may be seen from these observations, this type of degeneration of the fibroblast appears to be practically identical in nature with that described for the leucocyte under the influence of light.

Furthermore, it was found that these sensitized fibroblasts reacted to the red, the green and the blue components of the visible spectrum, and to the white light itself (the infra red and the ultra violet being blocked out), in a manner wholly comparable to that shown by the leucocytes.

This is a preliminary report.

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On the Experimental Production of Showers of "Non-Motile" Leucocytes.*

L. A. BEARD AND J. W. BEARD. (Introduced by R. S. Cunningham.)

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Schilling,[1] in 1908, while studying the degeneration of white blood cells under the dark field microscope, found that dying leucocytes showed a definite swelling of the nucleus, and a change in the size and refractivity of the specific granules. Similar changes were later described by Sabin[2] as taking place in the death of the polymorphonuclear neutrophilic leucocytes. In the latter case, these changes were observed while studying the white blood cells by means of the supra-vital technique. Sabin was able to follow every step in the change from the living, motile polymorphonuclear neutrophilic

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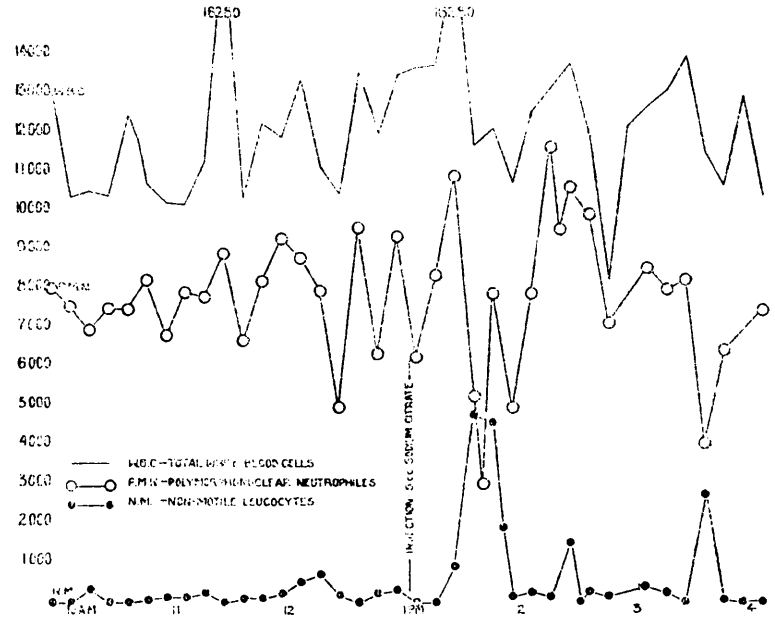
leucocytes to the dead, unstained cells, which she called "non-motile" leucocytes. She found that these changes could be produced by such technical means as pressure and heat, but after elimination of these errors she still found characteristic non-motile degenerating forms, and concluded that they represent a normal stage in the death and disintegration of the leucocytes. At the same time, she observed that these non-motile cells tended to appear in small showers, rather than in constant numbers. Later, in a detailed study of the normal rhythm of the white blood cells in the human, Sabin, Cunningham, Doan and Kindwall[3] confirmed the above observations concerning the appearance of non-motile cells in showers. They found in addition that these showers occurred at approximately hourly intervals.

In the course of a series of experiments in which we were studying the qualitative effect of sodium citrate, given intravenously, on the white blood cells, we were impressed by the large numbers of non-motile cells which regularly appeared following the administration of the citrate. A series of detailed experiments was then carried out to determine the exact character of these showers. It was found that the intravenous injection of a moderate amount of sodium citrate was uniformly followed by non-motile showers, which were many times greater than those seen in the normal rabbit. These showers regularly maintained the hourly rhythm, and were usually preceded by a definite clumping of the neutrophilic leucocytes. In several experiments the platelets were also counted, and were found to rise temporarily with some indication of a correlation with the showers of non-motiles. The supra-vital technique was used throughout the experiments for the differential counts.

The chart shows the results obtained in one of these experiments. The preliminary counts were made at 10 minute intervals over a period of about 3 hours, and during this time there were 3 small showers of 3 per cent, 2 per cent and 7 per cent respectively. During the 3 hours following the injection of 5 cc. of a 1 per cent solution of sodium citrate, 3 showers were obtained of 41 per cent, 18 per cent and 23 per cent; the striking finding being the maintenance of the normal hourly rhythm, with the great increase in the size of the individual showers.

In experiments in which comparatively larger doses were given, the normal rhythm was varied by the extension of the individual showers, so that there was a marked shortening of the intervals between them. Thus, some of the more extreme effects gave a marked instability of the curve, with non-motile cells present in



varying numbers for several hours following the injection. The amplitude of these showers of non-motile cells was uniformly far beyond that of any showers which we have observed in normal rabbits or of those reported by Sabin *et al.*[3]

By no technical or mechanical means has it been possible to induce such large numbers of degenerating leucocytes without producing general effects on other cells. This fact, taken together with the observation of the clumping of leucocytes in preparations preceding the shower of non-motile cells, with the admixture of normal active cells and non-motile cells in every preparation, and with the marked regularity with which these showers followed the injection of citrate, makes the conclusion seem unavoidable that these forms are produced within the circulation.

Any explanation of this phenomenon which may be advanced at the present time must be considered as only an hypothesis. It is possible that this great increase in non-motiles is the result of the injury to normal leucocytes, occurs either by direct action of the citrate on the cells, or indirectly through the action of the citrate on some mechanism capable of causing this effect in the organism. On the other hand, it is also possible that there is some storehouse where they may normally be formed or collected, from which they are expelled into the circulating blood by the action of sodium citrate on some mechanism, operating normally to produce the usual small

showers of these degenerated leucocytes which are found in the peripheral blood stream. Experiments are at present in progress to determine the exact nature of this mechanism.

This is a preliminary report.

[1] Schilling, V., *Fol. Haemat.*, 1908, vi, 429.

[2] Sabin, F. R., *Johns Hopkins Hosp. Bull.*, 1923, xxxiv, 277.

[3] Sabin, F. R., Cunningham, R. S., Doan, C. D., and Kindwall, J. A., *Johns Hopkins Hosp. Bull.*, 1925, xxxvii, 14.

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The Relation of Calcium to the Toxicity of Carbon Tetrachloride in Dogs.*

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In the treatment of several million cases of hookworm disease with carbon tetrachloride a few deaths have occurred. Some of these were probably due to the taking of alcohol with the drug, and others to mechanical obstruction by ascaris worms. In a very few instances, however, carbon tetrachloride was apparently the direct cause of death. In an attempt to explain these cases extensive studies have been carried out by Lamson and his associates.[1] These authors found that it was practically impossible to produce visible signs of intoxication in dogs by the oral administration of carbon tetrachloride in doses even up to the capacity of the stomach (250 cc.). Despite the general normal condition, pathological examinations and various functional tests showed that even single small doses caused considerable damage to the liver, while typical cirrhosis of the liver could be produced by repeated administration.[2, 3, 4, 5]

The work was transferred in 1925 to the Vanderbilt University Medical School. Continued studies here with various liver function tests gave results quite analogous to those reported in the earlier papers. The death of a great number of experimental animals, however, indicated that some new factor had been introduced in spite of the fact that the same carbon tetrachloride was used. In contrast to the earlier results, doses as low as 4 cc. per kilo body

* This investigation is one of a series of studies being made under the direction of Dr. P. D. Lamson on the pharmacology and toxicology of carbon tetrachloride. The work is being carried on with the support of the International Health Board.