FURTHER OBSERVATIONS ON THE INFLUENCE OF SALTS WHEN INJECTED INTO THE ANIMAL BODY.

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(Received for publication, November 22, 1922.)

In a previous communication (1) we reported the observation that following the exposure of rabbits to x-rays of relatively long wavelength their plasma bicarbonate is significantly increased. There was apparently some relation between the establishment of this state and the decrease in the circulating mononuclear cells known to follow characteristically after x-ray exposures. This observation led us to inject $NaHCO_3$, in solution, into rabbits and study what influence might be exerted on these same properties of the blood. The results were analogous to those following exposure to x-rays; namely, the pH was increased and there was a marked decrease in the circulating mononuclear cells. The maximum pH increase was reached in about 1 hour and the maximum decrease in the cells was reached in about 3 hours. This experience led us to investigate the effect of other salts when injected into the body. At the time the observations referred to were reported we did not have sufficient data on the latter experiments to warrant their publication. Since then we have carried our observations further.

We continued to use rabbits as experimental animals, and individuals of approximately the same weight (about 2 kilos) were selected. The conditions of feeding and the general care of the animals previously reported (1) were adhered to. Absolute and differential white blood cell counts were made in the usual way. Two or three counts were made before an experiment was begun and any animal whose count showed a significant fluctuation was not used. We believe that we have a sufficient number of standard counts to assure a satisfactory degree of reliability in the counts made following the injections of salt solution. In presenting the changes noted in the mononuclear

cells we have taken the number per cubic millimeter, observed before starting the experiment as 100 per cent, and subsequent counts are expressed in terms of percentage of the original number. Observations made following the injection of 5 cc. of 0.15 M NaCl revealed no significant change in the count. The same observation was made following the injection of 5 cc. of NaCl of the same concentration in 10 cc. volume.

We chose NaCl as the first salt to be injected. Since this is a neutral salt any result observed may be considered to be independent of pH change. The injection of 5 cc. of 2.4 M NaCl solution was followed by a marked drop in the number of circulating mononuclear cells per cubic millimeter of blood. 3 hours after the injection, the number of these cells was 70 per cent below the number present before the experiment was begun. After this time there was a gradual return to approximately the original count. At the end of 24 hours the blood picture was practically that observed before beginning the experiment. This procedure was repeated several times with similar results. This was true whether the injections were made intravenously or intraperitoneally. The same statement applies to all other results to be reported in this paper.

In order to investigate further the relation of pH to the changes observed, we injected solutions of Na_2HPO_4 and KH_2PO_4. Sörensen salts were employed and solutions of these were made equivalent with respect to the Na ion in the NaCl solutions; *i.e.*, 1.2 M Na_2HPO_4 and 2.4 M KH_2PO. It would be expected that some change in the pH of the blood would result but in opposite directions. The results observed were identical with those in the experiment where NaCl was injected, and seemed to rule out any influence of pH on the changes we had observed. These observations led us to believe that perhaps we were dealing with ion effects. This was suggested by the fact that the concentration of cations in all the solutions was the same. It was possible to test this idea further by injecting Na_2SO_4. We would expect that a 1.2 M solution of this salt would have the same effect as a 2.4 M solution of NaCl. This was found to be the case. It seems unlikely that altered osmotic conditions would play a rôle in the results observed, but in order to have direct information regarding any possible influence of this factor we injected solutions

of cane-sugar and solutions of glycerol. In each case solutions equiosmotic with 2.4 M NaCl were employed; *i.e.*, 4.8 M. We observed no significant change in the number of circulating mononuclear cells. The point of view stated, that we were dealing with ion effects, was encouraged by this observation which we accepted as evidence eliminating osmotic influences. The salts employed so far all furnish ions in common with those that are present in greatest abundance in the blood. It seemed desirable now to determine what effect would follow the injection of a salt with ions not found in significant amounts in the blood. A 2.4 M solution of $LiNO_3$ was injected, and results practically identical with those found in the cases of other salts were observed.

We now made injections of NaCl, $NaHCO_3$, and $LiNO_3$ in 1.2 M and 0.6 M solutions, and Na_2SO_4 in 0.6 M and 0.3 M solutions. All of these solutions influenced the blood picture in a similar manner; namely, the mononuclear elements showed a decrease of about 30 per cent instead of about 65 per cent as noted with the higher concentrations of the same salts. No significant difference was noted between the effect of either of these lower dilutions, and this suggests that there is a certain level in the concentration of ions which may be regarded as a threshold point.

Briefly stated, our observations are as follows: When NaCl, $NaHCO_3$, KH_2PO_4, $LiNO_3$, and Na_2SO_4, are injected into rabbits, of about the same weight, in solutions equivalent to 2.4 M NaCl, there is observed a marked decrease of approximately 70 per cent in the circulating mononuclear cells of the blood (Fig. 1). If the same salts are injected in solutions one-half and one-quarter this concentration, there is also a decrease in the number of the mononuclear cells, but only about half as great as is observed with the higher concentrations. Solutions of cane-sugar or glycerol equiosmotic with 2.4 M NaCl effect no significant change in the number of these cells. If only one injection of the salt in solution is made, the maximum decrease in the mononuclear cells is reached in about 3 hours, and 24 hours later the normal blood picture is observed. Since the results obtained are striking and uniform with all of the salts used, as long as the concentration of cations is equivalent, it is suggested that the changes observed are effected through the agency of the

cations. It is of interest to note that the cations employed belong to the same periodic group. Our experiments had to be discontinued

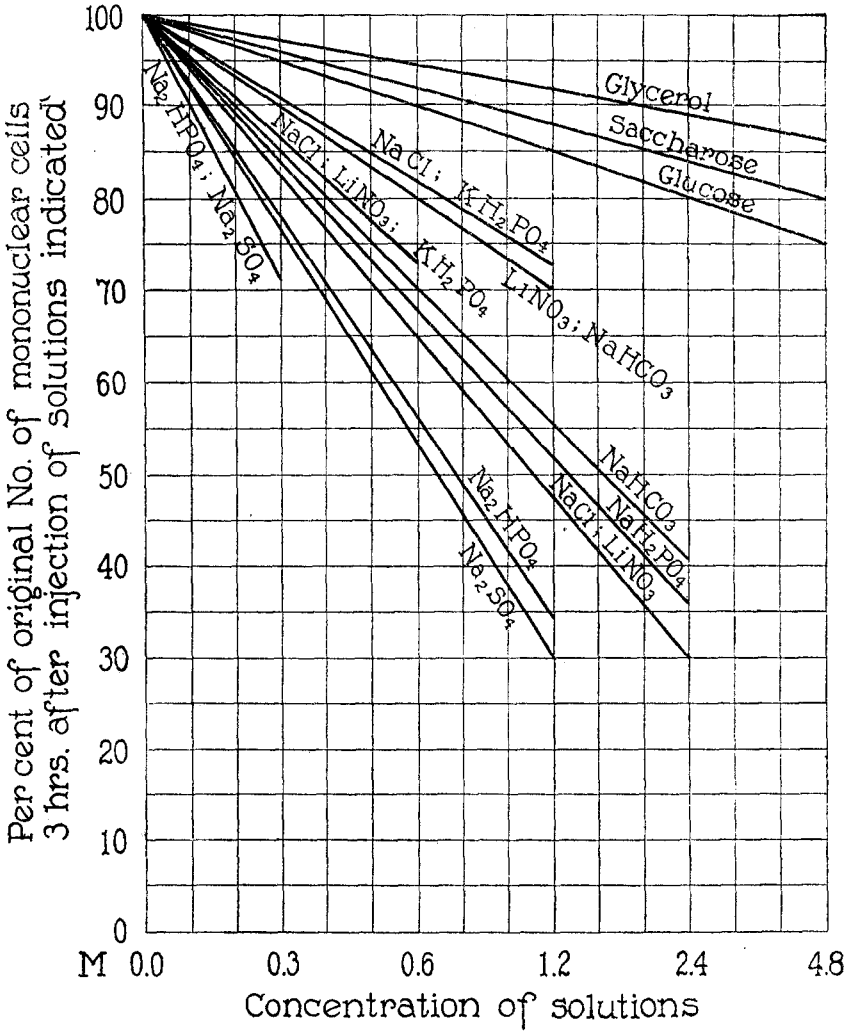

FIG. 1.

before it was possible to extend our observations to the effect of other cations and combinations of different cations, with reference to their antagonistic properties.

Our chief interest at present in the observations recorded centers about the analogy between the influence of the salts and the influence of x-radiations in so far as changes in the number of circulating mononuclear white blood cells is concerned. This analogy apparently increases in significance in the light of further observations made on animals where the injections of $NaHCO_3$ solutions were repeated several times. In these animals the decrease in the cells continues and the lymphoid tissue in the spleen shows evidence of definite injury analogous to the changes observed following x-ray exposure. The general health of the animal is not impaired in any observable manner. If this is true also in the case of repeated injections of other salts, as it would seem to be, it appears justifiable to assume that the changes are the result of a disturbance in the same chemical reaction. It does not follow, however, that the conditions leading to this disturbance are exactly the same. In fact the information we have relating to chemical changes brought about by x-rays indicates that these conditions are different. Our observations recall the experiences of Hertwig (2) who found that exposures of fertilized frogs' ova to radium not only retarded the developmental processes but that the larvæ hatched showed regular and characteristic changes. It was found later that the same retardation and subsequent changes could be induced by means of purely chemical agents. These results may be taken as further evidence of an end-result being affected by a disturbance in the same chemical reaction brought about through conditions entirely different. As a consequence of these experiences Hertwig stated that biological effects induced by radiation from radium were due to chemical reactions produced by them in protoplasm. This obviously is the case for both radium and x-rays.

CONCLUSION.

A satisfactory correlation of our observations dealing with the influence of salts and those dealing with the influence of x-rays is not possible at present. Any far reaching conclusion is not permitted because the information we have at this time regarding the physical chemical conditions concerned in the process of injury, as well as that pertaining to the nature of radio-chemical reactions, is too meager. As far as the experiments with salts are concerned, it may be

said that we are dealing with ion effects, and their importance in physiological processes is made clear by the investigations of Loeb (3) and those of Osterhout (4). The results that we have obtained in our experiments present an interesting analogy between the effect of x-rays and certain salts on the lymphoid elements of the animal body. We regard this analogy as significant in that it presents suggestions regarding the chemical nature of x-ray effects in the animal body.

BIBLIOGRAPHY.

1. Hussey, R. G., *J. Gen. Physiol.*, 1921–22, iv, 511.
2. Hertwig, O., *Arch. mikr. Anat.*, 1913, lxxxii, pt. 2, 1.
3. Loeb, J., *Am. J. Physiol.*, 1901–02, vi, 411; The mechanistic conception of life, Chicago, 1912.
4. Osterhout, W. J. V., *J. Gen. Physiol.*, 1920–21, iii, 611.