THE INFLUENCE OF X-RAYS ON THE PROPERTIES OF BLOOD.

By RAYMOND G. HUSSEY.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

(Received for publication, March 29, 1922.)

In the course of an experimental investigation concerning the nature of the reaction of the animal organism to x-rays we have made observations which we believe to be of sufficient interest to communicate in the form of a preliminary report. Our experiments were originally designed to throw some light on the question raised by the statement made by some roentgenologists that the characteristic illness frequently observed in individuals after an exposure to massive therapeutic doses of x-rays is due to a state of acidosis. The basis of this statement is apparently an empirical one since there is no rational evidence to support such an idea. We do not interpret the results of the experiments to be reported in this paper as a final answer to this question. We shall discuss this subject in a later paper.

Our procedure has been to study the numerical changes in the white cells of the blood, and the pH and bicarbonate content of the plasma in rabbits following an exposure of these animals to x-rays. Rabbits were chosen as the most convenient of the usual laboratory animals for our first observations on account of the facility with which they can be bled from the heart. We realize, however, that they are not ideal animals for our ultimate purpose. The plasma was obtained from blood drawn under paraffin oil into a tube which contained a sufficient amount of potassium oxalate to prevent coagulation. From 15 to 20 cc. of blood were drawn and the final oxalate concentration was about 0.3 per cent. Immediately after drawing the blood the plasma was separated from the cells by centrifuging. The surface of the oil was covered with low freezing point paraffin before centrifuging to prevent contact with air. We have found it possible to bleed a rabbit four or five times a week without any apparent effect on the

general reaction of the animal, as regards the conditions of our investigation. For the pH determinations we have employed the colorimetric method developed by Cullen,¹ and for determining the bicarbonate content we have employed the procedure of Van Slyke.² The pH determinations have been checked electrometrically as have also the standard buffer solutions used in the colorimetric tests.

Preliminary observations showed the range of the pH value of rabbit plasma to be between 7.12 and 7.26, values which are lower than those stated by Hasselbalch and Lundsgaard (1912), 7.33 (electrometric), and Kuriyama (1913), 7.4 to 7.5 (colorimetric). However, since the electrometric method employed in obtaining our values is more precise than was the method available at the time the values of Hasselbach and Lundsgaard were determined, we believe our values to be more nearly correct. We found the normal plasma bicarbonate content, expressed in volumes per cent of CO₂, to vary between 26 and 45. It is possible to obtain wide variations in a single rabbit unless careful attention is given to keeping the animal quiet during the process of bleeding. Feeding also plays a rôle in the variations of different samples of plasma but we have found it possible to control this factor by standardizing the amount of food and the time of feeding and the interval of time between the removal of animals from food before the beginning of the experiment.

The rabbits have been exposed to x-radiation from a Coolidge tube, the dosage factors being as follows: spark gap, 3 inches; current, 10 milliamperes; target distance, 6 inches; and time of exposure, 15 minutes. This is a massive dose of radiation of relatively long wave lengths, a large part of which are absorbed by the skin. This dose approaches the maximum amount that can be given without killing the animal and was purposely chosen as a starting point in order to obtain clear cut results. The exposures were made directly on the rabbit's abdomen which had been previously shaved.

EXPERIMENTAL.

1. Four rabbits were bled from the heart for plasma, then two of the animals were exposed to the radiation stated. The other two

¹ Cullen, G. E., *J. Biol. Chem.*, 1922, p. xvii.

² Van Slyke, D. D., *J. Biol. Chem.*, 1917, xxx, 347.

animals were not x-rayed. One control and one test animal were bled again 24 hours later and the other control and test animals were bled 48 hours after the x-ray exposure. 2 weeks later the test animals were bled again. The observations made on the plasma obtained at these times are given in Table I.

TABLE I.

Rabbit No.	Before radiation.		24 hrs. after radiation.		48 hrs. after radiation.		14 days after radiation.	
	pH	CO ₂ <i>vol. per cent</i>	pH	CO ₂ <i>vol. per cent</i>	pH	CO ₂ <i>vol. per cent</i>	pH	CO ₂ <i>vol. per cent</i>
1 (x-rayed.)	7.16	33.2	7.34	51.0			7.22	51.2
3 "	7.21	35.6			7.35	49.6	7.23	48.6
2 (Normal.)	7.20	35.2	7.15	33.5				
4 "	7.19	38.6			7.14	34.7		

This experiment was repeated with results similar in magnitude to those given.

2. Two rabbits were bled then x-rayed as stated. Blood was obtained from one of them an hour after the x-ray exposure, and from the other 3 hours after. The results of the observations on the plasma obtained are given in Table II.

TABLE II.

Before radiation.		1 hr. after radiation.		3 hrs. after radiation.	
pH	CO ₂ <i>vol. per cent</i>	pH	CO ₂ <i>vol. per cent</i>	pH	CO ₂ <i>vol. per cent</i>
7.26	38.5	7.30	48.0		
7.24	38.8.			7.35	54.6

In other experiments observations have been made on the plasma at various intervals following exposure to the x-rays and the above results corroborated.

Blood counts have been made on rabbits which were bled as well as x-rayed, and on others which were x-rayed only the changes do not differ significantly. When counts are made at 15 minute intervals the first decrease we have noted on the mononuclear elements occurs

$\frac{1}{2}$ hour after the x-ray exposure. After the first hour the interval between counts has been 1 hour and the maximum decrease occurs between 3 and 5 hours, the same period noted for the maximum change in the reaction of the plasma.

Summarized briefly the results of these experiments show that there exists a definite alkali excess in the body of the rabbit following an exposure to the dose of x-rays employed. Since there is an increase in the bicarbonate content of the plasma as well as an increase in the pH (*i.e.* a decrease in the CH^+), then evidently the $\frac{BA}{HA}$ ratio must be

disturbed in a manner which defines a state of uncompensated alkali excess.³ The maximum change in the reaction of the plasma is reached between 3 and 5 hours following exposure to radiation. The pH is observed to reach a normal level before the bicarbonate, which indicates the existence of a period of compensated alkali excess before the animal returns to normal. Evidence is found which significantly associates the point of maximum change in the reaction of the plasma with the maximum change noted in the white cell counts of the blood.

With these facts established we directed our attention to the possibility of producing a state of alkali excess in the rabbit by injecting sodium bicarbonate, and to the study of any chemical and morphological blood changes which might ensue. As a guide to the amount of salt to inject, we availed ourselves of the formula developed by Palmer and Van Slyke,⁴ for bicarbonate administration with reference to the effect of a given amount of salt on the reaction of the body fluids. The formula is based on the fact that 1 gm. of NaHCO_3 yields 267 cc. of CO_2 measured under standard conditions. If now it is assumed that 0.7 of the body weight is fluid, it is obvious that there are 700 cc. of fluid for each kilo of body weight and 1 gm. of bicarbonate would increase the CO_2 content $\frac{267}{7W}$ volumes per cent. This expression simplifies to $\frac{38}{W}$ where W is the body weight expressed in kilograms. If now we let b = the volume per cent increase in CO_2 , we

³ Van Slyke, D. D., *J. Biol. Chem.*, 1921, xlviii, 1.

⁴ Palmer, W. W., and Van Slyke, D. D., *J. Biol. Chem.*, 1917, xxxii, 499.

have the simple equation $b = \frac{38}{W}$. Obviously for g gm. of NaHCO_3 the equation becomes $g = \frac{bW}{38}$. As our results show, this equation permits one to calculate with a good degree of accuracy the change to be expected.

Four rabbits were bled and the bicarbonate content and pH of their plasma was determined. Later they were given an intraperitoneal injection of sodium bicarbonate in amounts stated and the reaction of their plasma was again determined after an interval of 1 hour with the results given in Table III.

TABLE III.

Rabbit No.	Weight.	NaH CO ₃ injected.	Before injection.		1 hr. after injection.			Difference between calculated and observed CO ₂
			pH	CO ₂	pH	CO ₂ observed.	CO ₂ calculated.	
	kilos	gm.		vol. per cent		vol. per cent	vol. per cent	vol. per cent
1	2.3	'	7.14	36.2	7.30	67.0	69.2	-2.2
2	2.3	3	7.19	35.6	7.42	88.1	85.2	+2.9
3	2.2	1	7.17	37.6	7.35	52.2	54.9	-2.7
4	2.1	1	7.22	40.6	7.36	55.4	59.9	-4.5

In other experiments similar observations have been made at varying intervals following injections of 1 gm. of bicarbonate. All injections were made into the peritoneal cavity. For periods after 1 hour the observed values are much less than the calculated values. The pH returns to normal in about 3 hours, but the bicarbonate content remains high up to 5 hours.

Observations on the white blood cells of these rabbits, as well as on other rabbits similarly treated show a diminution of white blood cells per cc. and the absolute counts show this to be due largely to the mononuclear forms. The maximum effect is noted 1 to 3 hours after the injection of the salt which time coincides with the period of maximum change in the reaction of the blood. This is true also in the rabbit following exposure to x-rays. Immediately following this maximum decrease there is a continued rise in the number of white blood cells per cc. and a rapid return to normal. It is possible, however, to maintain a low level of the mononuclear cells by repeated

injections of the salt. This is true in the guinea pig too, the only other animal to which we have so far extended these observations. Following an injection of 5 cc. of physiological saline solution we observe no significant change in the blood picture compared with blood counts on normal animals made at similar intervals.

DISCUSSION.

The facts thus presented develop an interesting analogy between the changes associated with x-ray effects and those found following injections of sodium bicarbonate. We do not feel justified at this time in attempting to make a definite interpretation of these results. The analogy, however, seems to be a striking one and it is very suggestive for further study, since it presents a possible opening for investigations concerning the mechanism of x-ray action on the animal body. Furthermore, evidence is found which justifies a doubt that the characteristic cell changes noted following x-ray exposures are due to the direct action of the x-rays. Additional force is given this doubt by the results of further experiments now in progress in which we are observing the effects accompanying the injection of other substances. So far we have used sodium chloride, sodium sulfate, monosodium phosphate, and glucose. Very definite changes are noted in the blood picture, but the data we have to date are not sufficient to permit of any further statement.

CONCLUSION.

When rabbits are exposed to x-radiation as described, there results a definite change in the $\frac{BA}{HA}$ ratio of their plasma. This change is evidently one which defines a state of uncompensated alkali excess.

The time required for a maximum change in the chemical reaction is the same as that required for a maximum change in the decrease of leucocytes.

Sodium bicarbonate injected into the peritoneal cavity is followed by results identical with those observed following exposure to x-rays. The maximum changes occur in a shorter time following bicarbonate injection, but the relation between chemical and morphological changes are the same.

We consider this analogy to be an important one in that it is suggestive of a relationship between the effect of salt and the effect of x-rays.