THE EFFECT OF ULTRA-VIOLET, X-RAY, AND RADIUM RADIATION ON THE TOXICITY OF NORMAL BLOOD.*

By DAVID I. MACHT and EBEN C. HILL.

(From the Laboratories of Pharmacology and Anatomy, Johns Hopkins University, Baltimore.)

(Received for publication, May 2, 1924.)

INTRODUCTION.

One of the authors in cooperation with various assistants has during the past few years been developing the new field of phytopharmacology, that is studying the effects of various drugs and poisons on plant tissues. This study has already yielded interesting results inasmuch as a comparison of the reactions of animal tissues to various drugs with the effect of the same substances on plant tissues gave very divergent findings. Thus, it was found that various substances which were very little, or not at all, toxic for zoopharmacological preparations were very toxic indeed for phytopharmacological preparations. For instance, in a study of cocaine and its decomposition products it was found that while the cocaine molecule was very toxic for animal protoplasm it was comparatively much less toxic for plant protoplasm (1). On the other hand, sodium benzoate which in small doses has no effect on animal tissues and is tolerated by animals even in very large doses, was very poisonous for certain seedlings which were studied. Again the phytopharmacological method was found to be especially adapted for the study of certain toxins such as menstrual toxin occurring in human blood (2). As a result of such observations it occurred to the authors that various changes in normal blood not ordinarily detectable by zoophysiological or zoopharmacological methods might be more easily detected by the use of phytophysiological or phytopharmaco-

* This investigation has been endowed in part by a grant from The Rockefeller Institute for Medical Research.

logical methods. In the present investigation the toxicity of normal blood for plant protoplasm was studied under normal conditions on the one hand and after radiation with rays of different wave lengths on the other hand.

Method.

The method employed was the same as that used by Macht and Livingston, and Macht and Lubin in other investigations. The effect of solutions of blood was studied on the growth of living seedlings of *Lupinus albus*. The procedure is described in detail in the above mentioned papers. Briefly, it consisted in growing a series of seedlings in a perfect nutrient medium such as Shive solution containing various salts necessary for the growth of the plants and comparing the growth of these plants with another series of seedlings suspended in a similar nutrient solution to which a small quantity of an unknown toxin or drug had been added. The increment in the growth of the well demarcated single straight rootlets can be easily measured and compared in such experiments. In the present investigation most of the experiments were made with defibrinated pig blood, a few experiments were also made with defibrinated ox blood, and still others were performed with human blood kept from coagulating by the addition of small quantities of heparin. Whenever an anticoagulant was used in studying blood, control experiments were made with normal nutrient solution containing the same concentration of the anticoagulant.

Experimental Results.

Effect of Ultra-Violet Radiation.—A series of experiments was made to determine the effect of ultra-violet radiation on the pharmacodynamic reactions of normal blood. This was found to be necessary in connection with a series of other investigations by one of the authors on the effects of carbon monoxide poisoning described elsewhere (3). The radiations were made with a Hanovia Alpine Sun mercury vapor quartz lamp by exposing 10 cc. of blood in an open glass dish, 5 cm. in diameter, at a distance of from 50 to 100 cm. Such radiations were given over a period ranging from 5 to 30 minutes. The toxicity of the blood after radiation was compared with the

toxicity of a sample of the same blood which had not been radiated by observing the effect of a 1 per cent solution in equal parts of distilled water and Shive solution on the growth of *Lupinus albus* seedlings.

The results obtained were quite definite and are exhibited in Table I. This table gives the *average* increment in the length of the roots of twenty seedlings for each experiment, at temperature of 22°C. at the end of 18 hours. It was found that ultra-violet radiation had no effect on the toxicity of blood for plant protoplasm and in some cases it actually rendered the blood less toxic.

Effect of X-Ray Radiation.—Many unrecorded experiments were necessary in considering the varied factors entering into the radiation of blood and blood serum. Results as to the length of exposure, the type of rays used, and comparison of Coolidge and gas tube rays had to be studied.

Tests of long exposure and low currents, shortened time with increased radiation, and almost instantaneous exposure (0.5 second) with a spark-gap at 10 inches and using 140 milliamperes were of interest and would justify many more experiments. As it is, however, this brief report does not justify more than very curtailed details. Soft rays did not penetrate to the depth of the fluid. Medium rays took about 2 minutes to affect the blood or serum so that the changes were demonstrable on the plant tissues.

The determination of any differences between rays from a gas or Coolidge tube demanded that both tubes be given great amounts of electric current. As a basis the spark-gap, the meter readings, and the results shown upon the same plate by radiographing the humerus were used. After setting the Coolidge and gas tubes at the points to give out the same milliamperage and spark control the gas tube was adjusted by a slight hardening or softening. A well seasoned nitrogen tube was used and little change had to be made. Then the Coolidge tube was adjusted, one half of an 8×10 plate was covered with lead, and the head and shaft of the humerus were radiographed on the unexposed portion by a gas tube. Immediately after this the lead plate was placed over the exposed portion of the plate and the Coolidge tube was used to radiograph the same bone. The time of exposure was the same. Development of the plate

TABLE I.

Growth of Lupinus Roots.

Experiment No.	Kind of blood.	Kind of radiation.	Normal growth.			Growth after radiation.		
			Whole blood.	Cells.	Plasma.	Whole blood.	Cells.	Plasma.
			mm.	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*
1	Pig.	Ultra-violet; quartz lamp.	7.6			7.6		
2	"	" " " "	10.3			10.3		
3	"	" " " "	18.7			18.5		
4	"	" " " "	12.9			13.0		
5	"	" " " "	9.9			10.0		
6	"	" " " "	8.5			8.5		
7	"	" " " "			9.0			8.9
8	"	" " " "	13.2			13.2		
9	"	" " " "	9.3			10.1		
10	"	" " " "		8.1	8.8		8.5	9.2
11	"	" " " "	10.3			10.3		
12	"	Radium emanations.	9.8			7.6		
13	"	" "	19.5			19.0		
14	"	" "	12.3			10.0		
15	"	" "		16.8	21.0		15.4	11.6
16	"	X-ray, gas tube.	10.4			8.5		
17	"	" " "	14.4			14.0		
18	"	" " "	20.6			18.8		
19	Ox.	" " "	15.6			15.2		
20	"	" " "	12.9			12.0		
21	Pig.	" " "	13.6			12.2		
22	"	" " "			12.0			10.6
23	Human.	" " "	18.0		20.4	17.4		18.8
24	Ox.	" " "	20.0		17.0	17.0		16.0
25	Pig.	" " "		17.6	19.1		15.4	16.6
26	"	" Coolidge "	16.0			12.4		
27	"	" " "	14.7			12.0		
28	"	" gas "	10.9			10.6		
29	"	" Coolidge "	10.9			9.1		
30	"	" gas "	12.3			10.8		
31	"	" Coolidge "	12.3			10.0		
32	"	" gas "		12.0	11.2		11.7	10.7
33	"	" Coolidge "		12.0	11.2		11.1	10.0
34	"	" gas "		5.0	5.6		4.7	3.6
35	"	" Coolidge "		5.0	5.6		4.2	3.2

indicated any differences in penetration and gave a fairly accurate idea of the quality of the rays. A decision of 2 minutes for radiation of the blood was partially empirical and depended to a certain extent on injury to the tubes by strain or over heating. By dividing this time of 2 minutes into three periods both tubes were uninjured in any way and being set aside for these experiments alone, needed little adjustment. Only once was it necessary to readjust a tube during radiation and in this instance a lead plate protected the blood.

The machine used was a powerful Wappler equipped for both gas and Coolidge tubes. The following standard was used.

Gas tube, seasoned, nitrogen.	Coolidge tube.
4 inch spark-gap.	4½ inch spark-gap.
55 kilowatts.	57 kilowatts.
30 milliamperes to tube.	30 milliamperes to tube.
54 cm. from center of focal point to surface of fluid. Focal point 2 mm.	4 volts used for heating filaments.
2 minutes time in periods of 40 seconds each.	2 minutes time in periods of 40 seconds each.

Controls in X-Ray Radiation.—In the experimental stage it appeared that pouring blood from a small Erlenmeyer flask to the dishes, radiating and then pouring back the fluid into the flask had some slight effect on the results obtained. So two or three dishes equal in size were filled to an equal depth. These were continually covered except during exposure. Any possible effect from secondary rays to the control was prevented by lead protection. The same precautions were maintained when one specimen of blood was to receive a Coolidge tube radiation and another specimen a gas tube radiation. Specimens radiated were immediately covered and placed with the control in a safe place. During the radiations the electric meters were carefully watched and later the tubes were tested for any marked changes if a target focal point had heated.

Effect of Radium.—A few experiments were performed by exposing samples of blood to radium emanations. Emanations of radium were obtained through the courtesy of Dr. Howard A. Kelly and Dr. Curtis F. Burnam. These emanations were contained in small glass pearls which were dropped into tubes containing 10 cc. of blood

and broken, after which the tubes were tightly stoppered with rubber stoppers. After exposure to the emanations for a period of 1 hour, the toxicity of the blood samples was compared with normal blood. Doses of from 5 to 8 millicuries were used.

Only a few experiments with radium were made but these definitely indicated that the effect produced on blood was very much the same as that obtained with Roentgen rays. The blood was rendered more toxic and the toxicity was chiefly confined to the plasma. Another striking feature in all the radium experiments was a distinct hemolysis produced in the blood.

SUMMARY.

While the number of experiments performed is perhaps somewhat limited the results obtained were quite definite and warrant the following conclusions. The toxicity of normal blood for living plant protoplasm as studied on the growth of *Lupinus albus* seedlings is definitely influenced by various radiations. Ultra-violet rays produce no effect on normal blood or may even render it slightly less toxic. Roentgen rays render normal blood more toxic. The toxicity is greater in the case of the blood plasma as compared with the blood cells and a more toxic effect is produced with the Coolidge tube as compared with the gas tube. Radium emanations in the few experiments performed produced changes very much the same as those given by the x-rays.

BIBLIOGRAPHY.

1. Macht, D. I., and Livingston, M. B., *J. Gen. Physiol.*, 1921–22, iv, 573.
2. Macht, D. I., and Lubin, D. S., *J. Pharmacol. and Exp. Therap.*, 1924, xxii, 413.
3. Macht, D. I., Blackman, S. S., Jr., and Kelly, E. B., *Proc. Soc. Exp. Biol. and Med.*, 1923–24, xxi, 289.