of alcohol and by great diminution of serological activity) along with the release of some reducing sugar or sugars. Whether this enzyme system of intestinal bacterial origin plays a part in the metabolism of dextran that has been injected intravenously in people remains to be settled.

THE TOXICITY OF P^{32} FOR NORMAL AND INFLUENZA VIRUS INFECTED EMBRYOS[1]

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In view of the increasing interest in the labeling of viruses with radioactive isotopes, it is of considerable importance to have some knowledge of the toxicity of these isotopes for chicken embryos. During an investigation conducted during 1948 and 1949 by Graham and McClelland (Can. J. Research, E, **28,** 121, 1950) on the uptake of P^{32} by influenza virus, some observations were made on the toxic effect of P^{32} in embryonated eggs. For 11 day old embryos, inoculated by the allantoic route with the PR8 strain of influenza virus A, the average number of deaths was found to be 3 to 4 per cent over a period of 48 hours. When P^{32} as inorganic phosphate, obtained from Chalk River, was injected by the same route shortly after virus, the embryo death rate amounted to as much as 30 per cent in 48 hours with 0.15 microcuries per egg. It was suggested that the deaths were caused by radiation damage although the possibility that some material toxic to the embryos might be present in the P^{32} was also discussed. In work conducted during the same period Taylor and Saenz (J. Immunol., **63,** 319, 331, 1949) injected up to 0.3 microcuries per egg, and Ward (Am. J. Hyg., **52,** 107, 1950) used up to 8 microcuries per embryo. Both groups of workers employed isotopes obtained from Oak Ridge.

Several months ago we were informed by Dr. Harvey Blank that his group at the Children's Hospital, Philadelphia, had conducted a study on the toxicity of P^{32} for embryonated eggs. Up to one millicurie per egg was used without deleterious effect, similar results being obtained with material from Oak Ridge and Chalk River. In view of the very large discrepancy between our results and the findings of the Philadelphia workers, we were led to reinvestigate this problem. The present paper describes an experiment designed to test the toxicity of P^{32}, currently produced at Chalk River, in both normal and PR8 virus infected eggs up to the level of 100 microcuries per egg.

The techniques were similar to those employed by Graham and McClelland (1950). P^{32}, obtained from Chalk River, as phosphoric acid, carrier free, was adjusted to pH 7 and sterilized at 100 C for 30 minutes. This was diluted with

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0.85 per cent sodium chloride buffered to pH 7 to give four solutions containing 107, 10.7, 1.07, and 0.1 microcuries P^{32} per 0.2 ml, respectively. Two-tenths ml of each P^{32} solution was injected into the allantoic sacs of a group of 40 11 day old chick embryos. Twenty eggs from each group were inoculated just previously with 0.2 ml PR8 virus by the allantoic route; the remaining twenty eggs from each group had received 0.2 ml buffered saline. Of a control group of 40 eggs, 20 were inoculated with 0.2 ml saline and the remainder received 0.2 ml PR8 virus per egg. Each egg of the control group was then injected with 0.2 ml saline. All eggs were candled twice daily and the deaths recorded (table 1). The allantoic fluids of all dead embryos were tested for sterility.

TABLE 1

Death rate of normal and influenza virus infected embryos after administration of varying amounts of P^{32}

GROUP	NUMBER OF DEATHS				TOTAL DEATHS IN 48 HOURS
	Hours after infection				
	17	24	41	48	
20 eggs + PR8 + 107 microcuries P^{32}	1	—	2*	—	3/20
20 eggs + saline + 107 microcuries P^{32}	—	—	2*	—	2/20
20 eggs + PR8 + 10.7 microcuries P^{32}	1	—	—	—	1/20
20 eggs + saline + 10.7 microcuries P^{32}	2	—	2†	1	5/20
20 eggs + PR8 + 1.1 microcuries P^{32}	1	—	—	—	1/20
20 eggs + saline + 1.1 microcuries P^{32}	2	—	—	—	2/20
20 eggs + PR8 + 0.1 microcuries P^{32}	—	—	—	—	0/20
20 eggs + saline + 0.1 microcuries P^{32}	—	—	—	1	1/20
20 eggs + PR8 + 0.2 ml saline	2	—	1	1	4/20
20 eggs + two injections of 0.2 ml saline	—	—	—	—	0/20

* Both deaths associated with bacterial contamination.

† One death associated with bacterial contamination.

Over the 48 hour period of observation, the death rate of normal or PR8 infected 11 day embryos injected with up to 107 microcuries P^{32} per embryo was not significantly increased over that of the controls. Although 5 from a total of 19 deaths could have been attributed to bacterial contamination, the number of deaths varied considerably from one group to another, including the controls, and cannot be related to any one responsible factor. It is concluded, therefore, that the high death rate observed previously with very much smaller amounts of P^{32} did not result from radiation damage but perhaps from the presence of some unknown toxic factor in the isotope.

It was found by Graham and McClelland (1950) that with less than 0.21 microcuries P^{32} per egg the specific activity of labeled virus rose in a linear manner with the amount of administered isotope. If this linear relationship extends to the 100 microcurie level, it should be feasible to obtain purified labeled influenza virus with specific activity at least 100 to 1,000-fold higher than that previously achieved.