nically satisfactory titrations have been accomplished using cells sent from a central source and treated according to the method described above.

Summary. It has been found feasible to ship large numbers of suspended HeLa cells packed in a small container. Neutralization titers of 185 sera tested with 2 different types of shipped HeLa cells have yielded reproducible results. Under the conditions described the neutralizing capacity of 4000 serums has been tested against 3 prototype strains of poliomyelitis virus in a color test.

‡ Virus pools were obtained from Connaught Laboratories. Control human and monkey antisera were obtained from Dr. Herbert A. Wenner, University of Kansas.

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Plasma Iodine^{131} Levels as Diagnostic Tests for Lethal X-Radiation Exposure in Rats. (22036)

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At present there is no single, simple, diagnostic laboratory test for lethal exposure of man or experimental animals to ionizing radiation. In reviews of clinical diagnosis and treatment of radiation injury, Dunham *et al.*(1) and Cronkite(2) have pointed out that determination of the sequence of occurrence of various signs and symptoms is the principal method for establishing the severity of radiation exposure in man. Of various clinical laboratory tests, the white blood cell count appears to be of most value for prognosis(1,2). Relatively few diagnostic tests have been reported for experimental animals. Rosenthal(3) found an opalescence in the serum of rabbits exposed to lethal doses of x-rays and reported a correlation with lethality. Entenman *et al.* (4) confirmed this finding in rabbits but were unable to demonstrate it in other species. These workers also determined plasma phospholipid phosphorus (PLP) levels in a number of species of experimental animals and concluded that though they might be of value for prognosis of mortality in the dog, the PLP level did not serve as a good indicator of radiation injury "since in all species lethal doses of irradiation (LD_{50} to LD_{100}) are required to produce appreciable changes." Hayes and Hewitt(5) separated the plasma lipids of irradiated rabbits into various "classes" by ultracentrifugation and found an excellent correlation between increases in certain classes and mortality. Their method, however, has the disadvantage of complexity and is probably not adaptable to routine use. The uptake of radioactive iron by red blood cells of irradiated animals is known to be well correlated with radiation dose(6-9). The apparent disadvantage to this method is, that while it is sensitive to relatively low dose ranges, the relationship does not hold well in the lethal ranges since maximum depression occurs at less than lethal levels. Gershon-Cohen and Hermel(10) have recently reinvestigated the very early changes in the white blood cell count of irradiated rats and have concluded that such information may be of value in prognosis.

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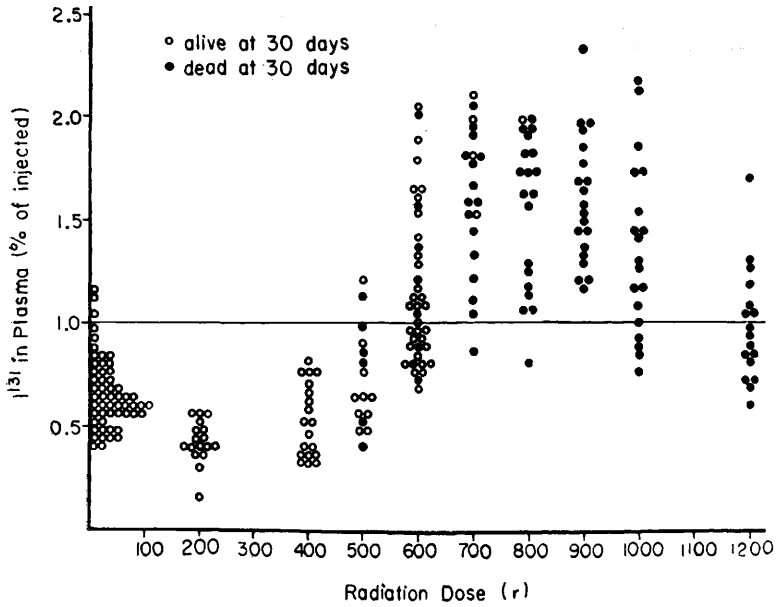

FIG. 1. Effect of various doses of x-radiation on iodine^{131} levels in the plasma of rats.

In the first of a series of projected studies on the distribution of fission products in irradiated rats it was noted that there appeared to be a relationship between plasma iodine^{131} level and the subsequent fate of the animal. This relationship was studied in more detail and is the subject of the present report.

Methods. A total of 242 adult male Sprague-Dawley rats was used. Since it was desirable to use as heterogeneous a group as possible, the rats selected weighed from 200 to 550 g and varied in age from approximately 3 to 18 months. They were housed 3 to a cage and given water and Purina Laboratory chow *ad libitum*. X-rays were delivered from a General Electric Maxitron unit using the following factors: 250 KVP, 30 ma, added filters of 1.0 mm Al and 0.5 mm Cu, TSD 50 cm, dose rate—115 r/min. The rats were exposed 3 at a time in a shallow lucite cage. The iodine^{131}, obtained from the Oak Ridge National Laboratory, was diluted with Krebs-Ringer phosphate buffer (pH 7) to a concentration of 5 μc/ml. Each rat received 1.0 of the solution intraperitoneally 48 hours following x-ray exposure. At that time suitable aliquots were set aside for counting at the same time that the plasma iodine determinations were made. Twenty-four hours after the injection of iodine (72 hours after irradiation), the animals were lightly anesthetized with ether and one ml of blood was removed into a heparin-wet syringe by cardiac puncture. The rats were allowed to recover, returned to their cages, and their survival was followed for 30 days. Autopsies were performed on animals dying the first few days following this procedure and any which showed evidence of hemorrhage from the puncture were excluded from the final tabulation of results. Blood obtained by cardiac puncture was placed in conical centrifuge tubes and the red cells were spun down. A 0.2-ml aliquot of plasma was then placed in a planchet containing 0.4 ml of 0.375 M NaOH and one drop of phenolphthalein solution. The solution was slowly evaporated to dryness under an infrared lamp and the iodine activity determined by beta counting under a thin-window Geiger-Muller tube connected to a conventional scaler. The iodine^{131} activity in the total plasma volume was calculated as per cent of the injected dose by the formula:

$$\frac{\text{(Counts/min. in 0.2 ml plasma)}\ \text{(body wt in g)}\ (14.725)}{\text{(Counts/min. in 1 ml of inj. sol.)}} = \%\ \text{of inj. dose in plasma.}$$

The factor 14.725 was obtained on the basis of the following considerations: (a) It was

TABLE I. Accuracy of Test in Prognosis at Various Doses of Radiation.

Radiation dose	No. rats	No. dead (30 days)	Positive values*				Negative values*				Combined accuracy, %
			Total	Correct	False	Accuracy, %	Total	Correct	False	Accuracy, %	
0	55	0	3	0	3	0	52	52	0	100	95
200	17	0	0	0	0	—	17	17	0	100	100
400	19	0	0	0	0	—	19	19	0	100	100
500	16	6	2	1	1	50	14	9	5	64	62
600	39	8	21	5	16	24	18	15	3	83	46
700	20	16	19	15	4	79	1	0	1	0	75
800	20	19	19	18	1	95	1	0	1	0	90
900	20	20	20	20	0	100	0	0	0	—	100
1000	19	19	14	14	0	100	5	0	5	0	74
1200	17	17	7	7	0	100	10	0	10	0	41

* Plasma I^{131} values in excess of 1% were considered "positive." Values of 1% or less were considered "negative."

assumed that there were 5.89 ml of blood/100 g body weight(8); (b) The hematocrit was assumed to be 0.50; (c) The counts/min. in the 0.2-ml sample of plasma were multiplied by 5 to convert to c/m/ml; (d) The entire equation was multiplied by 100 to convert to per cent,

$$\text{Thus: } \frac{5.89}{100} \times 0.50 \times 5 \times 100 = 14.725.$$

Results. Plasma levels of I^{131} (as per cent of injected dose) at various radiation doses are shown in Fig. 1. As radiation doses were increased in the range of 0 to about 900 r an increasing proportion of the rats showed relatively high levels of I^{131} in the plasma. At higher dose levels there appeared to be a reversal of the effect and the incidence of high values decreased.

Rats of the strain used in this study rarely die from radiation exposures of less than 500 r. At doses of 500 to 900 r, the incidence of lethality increases and at doses in excess of 900 r the incidence is nearly always 100%. The dose level of 500 r, then, can be taken as the point below which survival is reasonably certain and above which death may occur. From Fig. 1 it is apparent that I^{131} values of 1% or less were found in nearly all the control rats and rats exposed to less than 500 r. In the range of 500 to 900 r an increasingly greater percentage of the animals showed values in excess of 1%. For this reason, the 1% level was arbitrarily chosen to divide the results into "positive" (high) and "negative" (low) values. It was then assumed that positive values were prognostic of death and that negative values were prognostic of survival and the results were evaluated for accuracy as a possible diagnostic test. Table I lists the incidence of positive tests and lethality at various dose levels. In addition a tabulation of correct and incorrect predictions based on positive and negative I^{131} tests at each dose has been included. In this tabulation, death in a rat showing a positive value was considered a correct prediction as was survival in a rat showing a negative value. Death in rats showing negative tests or survival in rats showing positive tests were listed as false negatives or false positives, respectively. Percentage accuracy of positive and negative tests are shown separately and in the final column the over-all accuracy at each dose level is shown.

The test was not accurate at radiation doses in excess of 900 r, since a tendency to reversal of the effect occurred at these levels. This finding indicated that the test was valid over a restricted dose range and the doses in excess of 900 r exceeded this range. Accuracies at 1000 and 1200 r were computed in the same manner as for lower doses and, in the interest of completeness, are shown in Table I.

Discussion. Cronkite(2) has pointed out that for purposes of triage, a population exposed to ionizing radiation may be divided into the following groups: a. Sublethal exposure—recovery probable; b. Lethal exposure—recovery possible; c. Supralethal exposure—recovery improbable.

For purposes of evaluating the accuracy of the present test in dividing the sample rat population into these 3 groups, the population

was broken up as follows: a. Sublethal exposure—0 to 400 r; b. Lethal exposure—500 to 900 r; c. Supralethal exposure—over 900 r.

The assignment of the dosage ranges to these 3 groups was made arbitrarily based on the following considerations: Rats exposed to 0 to 400 r usually survive. In the range of 500 to 900 r some, but not all, of the rats usually die. With present methods of treatment of radiation injury, it is possible to increase survival in this range. Doses in excess of 900 r are uniformly fatal and survival is little influenced by present methods of therapy.

There was a total of 91 rats in the sublethal exposure group, all of which survived. Since 3 rats showed false positive values, the over-all accuracy in this range was 97%. Tests were run on 115 rats exposed in the lethal (500 to 900 r) range. Twenty-two rats showed false positive and 10 showed false negative values for an accuracy of 72%. The principal source of error in this dose range was in false predictions of death (false positives). Since such errors would not be serious if the test had been used as an indication for instituting therapy, it may be permissible to ignore them and consider only the false negatives as errors. In this case, the accuracy in the lethal range was 91%.

Only 2 doses in excess of 900 r were employed, namely, 1000 to 1200 r. These doses may properly be considered supralethal. Of the 36 rats tested, 21 showed positive values and 15 showed negative values. All the rats died. The accuracy in predicting death, then, was only 58%. The increased incidence of negative values in this dose range may be of value in triage. The diagnosis of severe radiation illness was obvious from the severe weight loss, diarrhea, ruffled fur, etc. With present methods of treatment of radiation illness, it would undoubtedly have been impossible to prevent death in these animals. The combined clinical impression of severe damage and a negative I^{131} test may facilitate the diagnosis of supralethal exposure. Further investigation of these and higher dosage levels is, of course, required.

If the values for accuracy at doses in excess of 900 r are excluded, then the over-all accuracy of the test in the 0 to 900 r range was 83 to 95%, depending on whether false-positive values are acceptable.

It must be emphasized that the test as employed in the present study was entirely empirical. The mechanism by which the plasma levels of I^{131} were increased is not known. Refinements in technic or the use of different time intervals might increase the accuracy. At present, it may be stated that under the conditions of the experiment it was possible to diagnose exposure to sublethal and lethal doses of x-radiation with fair accuracy over a restricted dose range.

Summary. By determining plasma levels of trace amounts of radio-iodine in rats 24 hours after injection and 72 hours after x-ir-radiation with 0 to 900 r, it was possible to divide the animals into sublethal and lethal exposure groups with an accuracy of 83 to 95%, depending on whether a false diagnosis of lethal exposure is acceptable. There appeared to be a reversal of the effect on I^{131} level at doses in excess of 900 r, which might be of value in triage. At these dose levels, the diagnosis of severe radiation injury was obvious from the appearance of the animals and the combined clinical impression of severe damage and a negative I^{131} test might facilitate the diagnosis of supralethal exposure.

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