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SECTION MEETINGS

CLEVELAND	
Institute of Pathology	December 8, 1950
Academy of Medicine	January 12, 1951
SOUTHERN	
Louisiana State University Medical School	December 15, 1950
SOUTHERN CALIFORNIA	
California Institute of Technology	January 11, 1951
WESTERN NEW YORK	
Syracuse University	January 13, 1951

Synergistic Effect of Phosphorus^{32} and Colloidal Gold^{198} on Survival in Male Albino Rats.* (18437)

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In recent years a great deal has been published on the survival of experimental animals after exposure to ionizing radiation. These studies include not only external beta and gamma rays but radiation from internally distributed radioactive elements. Having previously studied in this laboratory the survival rate of animals to the parenteral injection of P^{32}, Sr^{89}, and colloidal Au^{198} used individually, we now wish to present results of P^{32} and colloidal Au^{198} used together. Our combination experiments were directed primarily to the study of the simultaneous injury of separate body structures or systems and to the examination of their interrelationship in producing death of the animal. It was anticipated, if any clear cut results were to be obtained from these combination studies, that it would be necessary to utilize elements having widely different tissue distributions. Although P^{32} and colloidal Au^{198} are not ideal in this respect and Sr^{89} and colloidal Au^{198} would serve this purpose even better, this choice was dictated in part by the ease in preparation and handling of the isotopes. Colloidal Au^{198}† has a half-life of 2.73 days, emits negative electrons with a maximum energy of 0.97 Mev and one gamma ray with an energy of 0.411 Mev(1). When given intravenously to experimental animals(2,3),

* Work performed under Contract W31-109-eng-78 between the U. S. Atomic Energy Commission and Western Reserve University.

† Au^{198} was obtained in colloidal form from Dr. Tabern of Abbott Laboratories, North Chicago, Ill.

1. Mitchell, A. C. G., *Rev. Modern Physics,* 1950, v22, 36.

2. Block, Walter D., Buchanan, O. H., and Freyberg, R. H., *J. Pharm. and Exp. Therap.,* 1944, v82, 391.

3. Sheppard, C. W., Wells, E. B., Hahn, P. F., and Goodell, J. P. B., *J. Lab. and Clin. Med.,* 1947, v32, 274.

and humans(3), colloidal Au^{198} shows a high concentration in the liver, less in the spleen and bone marrow, and little or none in other organs(2). Because of the insolubility of metallic gold, blood levels are low(3) and excretion is less than 1% per day following its disposition in the body(6). We have found the 15 day LD_{50} for colloidal Au^{198} in male albino rats to be 35.5 ± 3 μc/g (S.D.) of body weight(4). P^{32} has a half-life of 14.3 days and emits negative electrons with a maximum energy of 1.71 Mev. When P^{32} is administered to experimental animals intraperitoneally, in the form of disodium phosphate (Na_2HPO_4), it appears in high concentration in bone and is also widely distributed throughout other organs in the body. The concentration of P^{32}, expressed in terms of the total injected dose, rises in the bone for the first 4 days following administration and then declines gradually. In most instances the percentage of injected dose of P^{32} in other organs reaches a maximum in less than 24 hours and then declines rapidly during the first 4 days to less than 20%. P^{32} in the form of Na_2HPO_4, unlike colloidal Au^{198}, is readily soluble and actively enters into the metabolism of all cells within the body. During the first 4 days, the rate of excretion of P^{32} from the body is a rapid one, probably representing a loss from the soft tissues, then followed by a more gradual loss, the rate of which is determined by the turnover of phosphorus in bone(4). We have found the 15 day LD_{50} for P^{32} in male albino rats to be 4.5 ± 0.2 μc/g (S.D.) of body weight(4).

Experimental procedure. The studies were divided into 2 groups. In the first group the combined effects of P^{32} and colloidal Au^{198} at both ends of the LD-scale were studied. Dosages were calculated by extrapolating to LD_{20} and LD_{80} from LD_{50} studies of these agents(4). After the results were obtained for studies at both ends of the scale, a second study was then arranged using dosages which theoretically should have fallen between the 2 previously chosen dosages. 252 male albino rats, (C. F. Wistar strain) of approximately the same age and weighing between 116-177 g, were used. The animals were divided by random selection into sub-groups as indicated in the tables. They were fed a diet of Friskies and water and were housed in separate cages in air-conditioned animal quarters. The P^{32}, which was obtained from the U.S. Atomic Energy Commission, Oak Ridge, Tenn., was adjusted to a pH of 7.0 with sodium hydroxide and its activity measured immediately prior to injection. All injections were given intraperitoneally and ranged between 0.1 and 0.4 cc per animal. The activity of the colloidal Au^{198} was measured immediately prior to injection and all injections were given intravenously. Following injection the animals were returned to their cages and observed for survival for a period of 20 days.

Results. The results of Groups I and II with the dosages and survival data are en-

TABLE I. Effect of Colloidal Au^{198} and P^{32} Used Singly and in Combination on Survival in Male Albino Rats.

Dosage	No. animals	No. dead	% dead Found	% dead Expected
Au^{198} 30 μc/g	24	3	13	—
P^{32} 3 μc/g	24	6	25	—
Au^{198} 15 P^{32} 1.5 μc/g	24	16	67	19
Au^{198} 40 μc/g	24	10	42	—
P^{32} 4.6 μc/g	24	15	63	—
Au^{198} 20 P^{32} 2.3 μc/g	24	23	96	52

TABLE II. Effect of Colloidal Au^{198} and P^{32} Used Singly and in Combination on Survival in Male Albino Rats.

Dosage	No. animals	No. dead	% dead Found	% dead Expected
Au^{198} 30 μc/g	12	1	8	—
P^{32} 3.3 μc/g	12	3	25	—
Au^{198} 15 P^{32} 1.65 μc/g	12	11	92	17
Au^{198} 34 μc/g	12	8	67	—
P^{32} 3.7 μc/g	12	7	58	—
Au^{198} 17 P^{32} 1.85 μc/g	12	12	100	68
Au^{198} 38 μc/g	12	11	92	—
P^{32} 4.2 μc/g	12	8	67	—
Au^{198} 19 P^{32} 2.1 μc/g	12	12	100	78

4. Bonte, F. J., Storaasli, J. P., and Friedell, H. L., Unpublished data.

tered in Table I and II respectively.

Discussion. Colloidal Au^{198} and P^{32} when used in combination have a clear cut synergistic effect in producing death in the albino rat. Doses of each which individually produce a low death rate, result in a markedly increased mortality when administered in combination. Theoretically, if complete additivity exists, half of a definitive dose of radiation A plus half of a definitive dose of radiation B should give an effect equal to a definitive dose of A or B used singly. As may be seen in Table I, dosages of $\frac{30}{2}$ μc/g of colloidal Au^{198} and $\frac{3.0}{2}$ μc/g of P^{32} which on a purely additive basis should have resulted in death of 19% of the animals, actually produced death in 67%. Zirkle(5) tabulated the available data on various types of radiation administered in combination and noted that they were either incompletely or completely additive and that no instance of synergism had been observed. The studies presented here do not actually conflict with Zirkle's data, since the experiments with which he concerns himself are those in which the radiations are dissimilar but the tissues irradiated are the same. Here we present information in which essentially the same radiations are administered in combination, but the distribution of the radiations in tissues is different. In making reference to synergism, we refer to the biological effect on the total organism rather than the biological effect on any specific tissue.

The potentiating effect appears to be decreased at the upper end of the survival curve. This apparent decrease at the upper levels is due to the fact that for all combinations utilizing doses above the LD_{50}, the curve is sharply compressed by the limiting LD_{100}. At the present time there is nothing to indicate that the variation in energies or half-lives is related to the observed synergism.

As suggested previously, the most important difference and probably the clue to the potentiation is the difference in distribution of these two agents. Colloidal Au^{198} when given intravenously is removed from the circulation by the cells of the reticuloendothelial system. Though these cells are widely distributed, the bulk of the colloidal Au^{198} is removed by the liver (Kupffer cells), spleen, and bone marrow. P^{32}, though widely distributed at first, is retained most heavily in bone and bone marrow.

When these two agents are given in combination the reticuloendothelial system and the bone marrow with their specialized functions are simultaneously injured. The interrelation between these two systems at present is obscure but some speculation is justified in view of the findings by Jacobson(6). He has shown that protection of the spleen during radiation of the animal results in a sparing effect on the hematopoietic system and a correspondingly greater survival rate. It is attractive to postulate that injury to the

TABLE III. Energy Calculations in Ergs per g of Tissue* with LD_{50} Doses of P^{32} and Colloidal Au^{198}.

			Combination	
			Au^{198}	P^{32}†
	P^{32}	Au^{198}	2	2
Femur‡	9.58×10^5	0.717×10^5	5.149×10^5	
Spleen	1.48×10^5	5.25×10^5	3.37×10^5	
Liver	1.36×10^5	35.9×10^5	18.63×10^5	

* Calculations were made on distribution data obtained on the third day following injection of colloidal Au^{198} and P^{32}.

† It is assumed that the distribution of P^{32} and colloidal Au^{198} will not be varied when these agents are used in combination.

‡ Bone and bone marrow are included together in determining percent of injected dose per gram of tissue in bone.

Energy calculations were made using the following formula:

$D = MP\ (1\text{-}C_{15})\ (2.22 \times 10^6)\ (1.44 \times T_b)\ E\ (1.6 \times 10^6)$ where:

D = ergs per g of tissue.
M = total LD_{50} dose in μc.
P = %/g of total inj. dose in organ.
C_{15} = decay factor for 15 days.
2.22×10^6 = disintegrations/min./μc.
$1.44 \times T_b$ = biological average life in minutes.
E = average beta energy in Mev.
1.6×10^6 = ergs per Mev.

5. Zirkle, R. E., *Am. J. Roentgenol.*, 1950, v63, 170.

6. Jacobson, L. O., Simmons, E. L., Bethard, W. F., Marks, E. K., and Robson, M. J., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 455.

spleen and other parts of the reticuloendothelial system aggravates the injury to the hematopoietic system. A dose ordinarily producing mild injury to the blood forming system evokes, therefore, a severe depression of the blood forming tissues and an increased death rate.

Analysis of energy calculations (energy absorbed per gram of tissue) will probably not be fruitful at this time since the biological effect is dependent not only upon the radiation dosage but upon the radiosensitivity of the tissues. In addition, a great deal of information can be obtained by future experiments. However, these calculations were made and are entered in Table III. It may be seen that the liver and its reticuloendothelial system is very intensively irradiated when Au^{198} singly or Au^{198} and P^{32} combined are given. This would suggest that the liver injury is an important factor in producing the increased death rate.

With doses at the LD_{50} level, the spleen and bone marrow are severely damaged histologically by both colloidal Au^{198} and P^{32}. The liver, however, is only slightly damaged by P^{32} alone while seriously damaged by colloidal Au^{198}.

Summary. Data have been presented on the survival of the albino rat receiving radiation from internally administered P^{32} and colloidal Au^{198}. These isotopes have been administered singly and in combination. The data clearly indicate that P^{32} and colloidal Au^{198} when administered in combination act synergistically in their killing effect. This appears to be related to the simultaneous injury of the reticuloendothelial system and the blood-forming tissues.

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Further Studies on Human Hetero-Hemagglutinins for Mouse Erythrocytes.* (18438)

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Natural agglutinins for mouse erythrocytes occurring in human sera have been studied by Gorer(1), and Cohen, Winokur, Kuhns and Figge(2). Gorer(1), using Blood Group A serum, was able to demonstrate antigenic differences in mice by the use of agglutination and adsorption technics. Cohen *et al.*(2) reported that the presence or absence of the "Mo agglutinin," the term they used to designate this hemagglutinin, seemed to divide human sera into two groups independent of their major blood groups. Since only 37 individual sera were studied in the previous work(2) a more extensive investigation was undertaken to obtain more accurate knowledge of the distribution of the Mo agglutinin. This extended investigation has demonstrated that naturally occurring hemagglutinins for mouse erythrocytes are present in all human sera tested. The previous failure to demonstrate this agglutinin in all sera(2) probably resulted from the use of a technic which did not detect positive reactions in sera with low agglutinin titers.

Materials and methods. Four hundred human blood samples were obtained from the Baltimore Rh-Typing Laboratory and the Blood Bank of the University Hospital, Baltimore, Md.† The blood was allowed to clot

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1. Gorer, P. A., *J. Gen.*, 1936, v32, 17.

2. Cohen, L., Winokur, G., Kuhns, W. J., Figge, F. H. J., PROC. SOC. EXP. BIOL. AND MED., 1948, v67, 548.