Labeling of Rabbit Platelets with Iodine^{131}. (20902)

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Mueller(1) has demonstrated that rabbit platelets can be labeled satisfactorily with radioactive phosphorus (P^{32}), but that the labeled platelets disappear rapidly from the blood on reinjection into rabbits. From the results of *in vitro* studies he concluded that the platelets were injured in the process of labeling and preparation for reinjection. Julliard(2) also used radiophosphorus to label platelets, with similar unsatisfactory results. Because of the successful use of iodine^{131} in protein labeling, it was decided to attempt to label platelets with radioactive iodine, in the expectation that the protein portion of the platelet might demonstrate similar avidity for iodine.

Materials and methods. 1. *Obtaining of platelets.* Rabbits' heart blood was drawn using 3.8% sodium citrate as anticoagulant and wash fluid in Exp. 1-4, and 10% sequestrene (disodium salt of ethylene diamine tetra-acetic acid) as anticoagulant and 0.85% saline as wash fluid in Exp. 5-13. In Exp. 9-15, 2% triton WR 1339† was utilized as described by Minor and Burnett(3) to minimize agglutination. High molecular weight dextran‡ was used to separate the red cells in clot retraction studies. All needles, syringes and glassware were siliconized prior to use. Platelets were concentrated and washed free of plasma proteins by differential centrifugation of blood and plasma(3) and washed twice with 10 ml of citrate or saline. The final volume of the platelet suspension was 1-2 ml. 2. *Radioiodination.* To the platelet suspension in a siliconized tube immersed in ice was added 0.05-0.10 ml of 0.089 M iodine in 0.1 M potassium iodide, and 100-200 μc of iodine^{131} in 0.05-0.20 ml, all buffered to pH 8.0 with boric acid-sodium hydroxide buffer(4). Exposure to iodine was terminated after 15 minutes by adding 1-3 drops of 0.1 M sodium thiosulfate. The material was centrifuged immediately at 2500 rpm for 30 minutes. The platelet sediment was washed 3 times by resuspension in citrate or saline, and recentrifugation. Determinations of radioactivity of dilutions of washings and of platelets were performed in duplicate using one ml aliquot samples dried in steel cups and mounted 3 cm beneath an end window Geiger-Müller tube (mica 1.4 mg/cm^2).

Platelet counts were done in duplicate using red cell pipettes, and diluting with Rees-Ecker(5) fluid. Standard hemocytometer chambers were used. In instances where platelet labeling was adequate (G-M counts totaling over 10,000 cpm) the platelets were resuspended in 5-10 ml plasma and injected into the rabbit during a 5-minute period by cardiac puncture. Ten ml cardiac blood samples were withdrawn 5 minutes, one hour and 2 hours after injection, and the platelets separated by differential centrifugation and one citrate or saline wash. Radioactivity determinations were made on the dried platelets as well as on plasma and wash materials. 3. *Clot retraction studies.* Qualitative clot retraction tests were done in 12 x 100 mm non-siliconized glass test tubes with one ml platelet-poor plasma (prepared by centrifuging sequestrene-treated blood for 2 hours at 2500 rpm), 2 ml 0.01 M calcium chloride, and aliquots of the platelet preparations in volume sufficient to provide 10,000,000 platelets per tube (*e.g.* total volume 3.0-3.4 ml). The plasma-platelet-calcium mixtures were incubated at 37°C for one-half hour following clot formation, and preliminary readings were made. Tubes were stored 18 hours in the icebox before final readings were made. The controls for the procedure consisted of platelet-rich plasma (or plasma from which only red cells were removed by dextran sedimentation) and platelet-poor plasma, prepared as

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† Triton WR 1339 kindly supplied by Rohm and Haas, Philadelphia, Pa.

‡ High molecular weight dextan generously supplied by Dr. Homer E. Stavely, Commercial Solvents Corporation, Terre Haute, Ind.

TABLE I. Radioactivity and Numbers of Rabbit Platelets Labeled with I^{131}.

Exp. No.	Total platelet count before iodination	Total platelet count after iodination	Total iodinating fluid radioactivity, cpm	3rd wash, cpm/ml	Total platelet radioactivity, cpm	% Tag†
1	1.4×10^9	2.0×10^8	6.7×10^6	1.7×10^3	1.1×10^5	1.22
2	3.2×10^8	$1.3 \times$ "	$5.5 \times$ "	8.0×10^2	1.2×10^4	.19
3	$6.6 \times$ "	$3.1 \times$ "	$4.9 \times$ "	2.6×10^4	$3.9 \times$ "	1.6
4	1.6×10^9	$2.4 \times$ "	$5.0 \times$ "	3.4×10^2	$3.9 \times$ "	1.6
5	$5.1 \times$ "	$8.3 \times$ "	$4.5 \times$ "	5.6×10^3	$1.6 \times$ "	.3
6	2.5×10^{10}	$4.2 \times$ "	$5.0 \times$ "	5.0×10^2	$2.7 \times$ "	.5
7	$1.1 \times$ "	—	$5.5 \times$ "	$2.7 \times$ "	$1.5 \times$ "	.3
8	4.2×10^9	$7.9 \times$ "	$5.6 \times$ "	$2.2 \times$ "	$2.7 \times$ "	.5
9	$3.2 \times$ "	1.2×10^9	$5.2 \times$ "	$1.3 \times$ "	$2.9 \times$ "	.3
10	$3.7 \times$ "	8.4×10^8	$8.2 \times$ "	$2.6 \times$ "	$5.7 \times$ "	.8
11	$5.1 \times$ "	1.5×10^9	$9.0 \times$ "	2.6×10^3	$1.3 \times$ "	.1
12	$9.7 \times$ "	—	$7.0 \times$ "	8.2×10^2	4.5×10^3	.1
13	$6.8 \times$ "	3.7×10^8	1.2×10^7	$7.5 \times$ "	2.6×10^4	.2
14	—	—	3.0×10^6	1.1×10^3	3.5×10^3	—
15	—	—	$9.7 \times$ "	$4.7 \times$ " *	$3.7 \times$ "	—

* Washing continued to 5 times with 5.4×10^2 cpm/ml on last wash.

† $\frac{\text{Platelet radioactivity}}{\text{Added radioactivity}} \times 100$.

previously described. Platelet-rich plasma on recalcification showed 4 plus clot retraction in 30 minutes, whereas platelet-poor plasma showed negligible clot retraction after 18 hours.

Results. Table I shows total platelet counts before and after iodination, total platelet-bound radioactivity counts and wash radioactivity counts in cpm/ml for 15 radioiodinations. As shown in the table an appreciable *in vitro* tag of the platelets with I^{131} was obtained. Total platelet counts ranged from 3.2×10^8 to 2.5×10^{10} prior to iodination, and from 1.3×10^8 to 1.5×10^9 after iodination, washing and centrifugation. In Table II are listed the radioactivity counts of plasma and platelets withdrawn from 5 rabbits, 5 minutes, one hour and 2 hours after the injection of tagged platelets. It is apparent from the rapid disappearance of the platelet-bound radioactivity and the subsequent rise in plasma radioactivity of the blood that the injected platelets do not survive and may be presumed to be defective.

TABLE II. Radioactivity of Plasma and Platelet Fractions of Rabbit Blood following Reinjection of I^{131} Labeled Platelets.

Exp. No.	Platelet radioactivity inj., cpm	Time after inj.	Radioactivity in 10 ml blood samples, cpm: Total plasma fraction	Total platelet fraction
6	2.7×10^4	5 min.	11	2
		1 hr	12	2
8	2.7×10^4	5 min.	16	8
		1 hr	79	11
9	2.9×10^4	5 min.	22	40
		1 hr	71	0
10	5.7×10^4	5 min.	40	7
		1 hr	98	0
13	2.6×10^4	5 min.	66	2
		1 hr	85	4
		2 hr	31	0

To determine the stage of the procedure at which this damage occurred, platelets were tested for their ability to restore clot-retraction to platelet-poor plasma after each step of the multiple procedures of centrifugation, washing, and chemical exposure, both radioactive and non-radioactive. It was apparent from these studies that multiple centrifugation and washing of platelets in citrate or saline impaired their ability to promote clot retraction.

Tagging of unwashed platelets, in the presence of small amounts of plasma was attempted on 2 occasions (Exp. 14 and 15) but, as anticipated, in the presence of plasma proteins the amount of radioiodine taken up by the platelets was insignificant, and inadequate for use in reinjection technics.

Summary. 1. When separated from plasma

proteins, rabbit platelets could be labeled adequately *in vitro* with radioiodine. 2. Platelets thus labeled *in vitro* did not promote clot retraction and disappeared rapidly from the circulation on reinjection. 3. The probable reason for the failure of the tagged platelets to survive following reinjection is injury to the platelets, resulting from the separation and concentration technics. 4. Since separation of platelets from plasma proteins is essential for I^{131} labeling, this method does not appear adaptable to studies of the *in vivo* metabolism of normal platelets.

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Glucocorticoids in Adrenocortical Carcinoma of Mice. (20903)

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Adrenocortical hyperplasia or carcinoma is observed in certain inbred strains of mice following early castration(1,2). In guinea pigs a nodular hyperplasia of the adrenal cortex following castration has been reported (3). Both the hyperplastic and the cancerous adrenals have been shown to secrete sex hormones, as evidenced by changes in the uterus and vagina(4), the prostate and seminal vesicles(5,3), the submaxillary gland and kidney(6) and the breast(4). Urinary secretion of estrogens has been studied in ovariectomized mice with adrenocortical tumors(7). Dickie and Woolley have studied the pituitary changes, especially with regard to the basophilic cells, in mice with postcastration adrenocortical cancers; they suggest that the syndrome in mice may well be an animal counterpart of Cushing's syndrome in man. Whereas no cell types in the mouse pituitaries were identified as exact counterparts of Crooke's changes, certain observed cell changes were considered in some respects similar to those described by Crooke, which in man are characteristic of Cushing's syndrome (8). Recent observations concerning the occurrences of Crooke's changes in individuals treated with cortisone suggest that in Cushing's syndrome the Crooke's changes are the result of the excessive secretion of C_{11}-C_{17} oxygenated adrenal steroids(9,10). In the mouse, evidence has been presented heretofore only for sex steroid secretion by these adrenocortical carcinomas. It therefore appeared desirable to examine them for the presence of C_{11} oxygenated steroids.

Materials and methods. Adrenal cancers were induced in Minneapolis (a) in F_1 hybrids of AxCE mice and transplanted into hybrids as described elsewhere(11) (first transplant generation from 3 primary tumors), and (b) in CE mice, with transplantation into the same strain (pool second transplant generation from 4 primary tumors). The mice were shipped to Philadelphia, where the tumors were harvested when they had reached an appropriate size. The tumors were dissected out, weighed and homogenized in a Potter homogenizer. The homogenate was used for bioassay of "corticoids", by the mouse liver glycogen method of Venning(12). The tissue homogenate was mixed directly with the glucose-alcohol in some instances; in others an acetone extract was prepared, and the dried extract dissolved in alcohol and added to the glucose. In one experiment, 30

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