more experimental rat retrieved only after 72 hours. There was no observable difference in the nature of the retrieving behavior of the normally-delivered mothers and the caesarean cases.

These results suggest that the process of giving birth and associated activities such as cleaning the young and eating the placenta are not essential precursors to post-parturitional nest building and caring for the young. The fact that 3 of the experimental females failed to display maternal behavior may have been due to one or all of several factors. They may have suffered more severe shock as a result of the operation than did the remaining 7 rats in this group. They may have been resistant to the stimulus of foster young in the retrieving tests. Or they may have been less "maternal" even before the operation. It has been reported that some otherwise normal female rats will not build nests or care for their young (Beach,1937). The 3 negative experimental animals in the present experiment may belong in such a category. Their failure to build nests prior to operation is evidence in favor of such an interpretation.

Regardless of the explanation for the 3 non-maternal animals, the occurrence of normal nest-building and retrieving in 7 experimental cases supports the conclusion advanced above. Furthermore, since the experimental females probably did not lactate normally it can be added provisionally that secretion of milk and nursing are not necessary for maternal responsiveness. Since mere contact with new-born rats did not evoke maternal reactions in the nulliparous controls it appears probable that the key factors involved in maternality are the hormonal changes associated with pregnancy and its termination.

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Pathologic Physiology of Mammalian Blood Platelet Utilizing P^{32} Tagged Rabbit Platelets.* (20417)

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Recently much attention has been focused on the etiology and pathologic-physiology of thrombocytopenia. One of the aspects studied has been the life span of the normal platelet in patients with various types of thrombocytopenia. This was accomplished by transfusing platelet-rich polycythemic blood into persons with thrombocytopenia. The work was begun in an effort to perfect a method for the study of platelet survival in the rabbit prior to attempting a similar study in human beings. During the course of the investigation some interesting observations concerning the physiology of the platelet have been made. In addition, a new technic is described which may be of value in future studies of this cell.

Weisberger and Heinle(1) have utilized the technic of isotopic labelling to study the survival rate of transfused leukocytes. A similar procedure has been devised for the platelet utilizing radioactive phosphorous. Julliard (2) and his co-workers in France have very recently published some data on the fate of transfused radioactive platelets. Their results are comparable to those herein reported.

Methods and results. Unagglutinated platelet suspension, essentially free from erythrocytes and leukocytes are prepared in the following manner. Silicon technic is used throughout the procedure. 45 cc of rabbit heart blood collected in 5 cc of Na Sequestrene is centrifuged at 800 rpm for 15 minutes. The platelet rich plasma is then transferred to

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especially manufactured platelet isolation tubes.† These are wide-mouthed, narrow-stemmed tubes which allow the buffy coat to be extracted readily with capillary pipettes. The tubes are spun, in the cold, at 3000 rpm for 20 minutes. The platelet layer is removed, resuspended in cold saline and centrifuged again. Usually 3-4 such centrifugations suffice. The final product is suspended in 5 cc of cold saline. With this method a 40-60% yield is considered satisfactory, since a considerable loss is necessary to assure purification. Fifteen-twenty microcuries of inorganic radioactive phosphorus is then added to the platelet suspension. This preparation is gently agitated at 37°C overnight. The following morning the platelets are washed 3 times with cold saline, again using high speed centrifugation in the cold. The platelets which are now labeled with radioactive phosphorus are finally suspended in 5 cc of cold saline and are ready for whatever testing is to be done. The platelet count of the final preparation averages 500000 to 1000000 platelets per cmm. Aliquot specimens of each wash water as well as the final specimen are taken for radioactive counting. All radioactive counting is done using a Geiger-Mueller counter with a mica end window and is recorded as beta counts per minute per given amount of specimen. As stated above aliquots were always saved so that no correction was necessary for decay. Originally the platelets were tagged *in vivo* by injecting inorganic radioactive phosphorus intravenously in rabbits and then removing the platelets after 48-72 hours. However the magnitude of the activity acquired by the platelets with such a procedure was so low that *in vitro* methods were necessary. This can be appreciated by referring to Table I. The difference between the two methods is obvious, as well as the fact that the platelets are capable of incorporating considerable radioactivity.

TABLE I. Example of Typical Experiment Showing that Platelets Readily Incorporate Radioactivity after *In vitro* Incubation with P^{32} as Compared to *In vivo* Tagging.

Specimen	Counts/min./1 ml	
	In vitro tagging	*In vivo* tagging
1st wash	21644	795
2nd "	8744	32
3rd "	3464	16
Platelet suspension	105284	72

† Obtained from E. Machlett, 220 E. 23rd St., New York City.

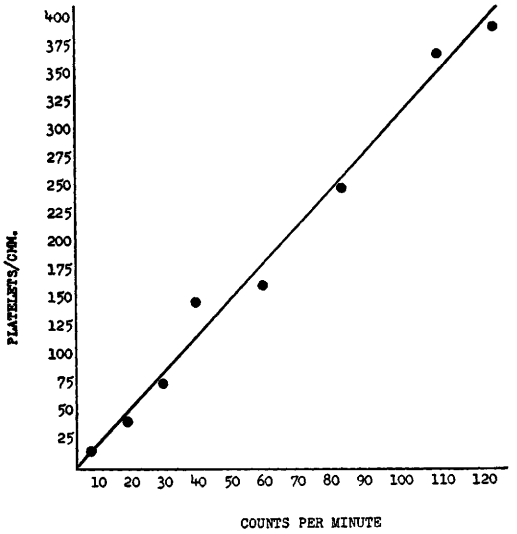

FIG. 1. Correlation between absolute platelet counts and radioactivity.

Results. In an attempt to demonstrate specificity of activity in the platelets tagged *in vitro* the radioactive count of such a preparation was correlated with decreasing numbers of platelets. A direct relationship is present as is shown in Fig. 1. Furthermore the radioactivity of the wash water levels off after 3 washings and ordinarily contains less than 5% of the activity present in the platelets.

For an isotopic labelling technic to be valid one must not only prove specificity of activity but also demonstrate incorporation of the P^{32} into the cell. If overnight *in vitro* incubation dislodges the radioactivity from the platelets the use of such a technic for studying survival rates is hampered seriously. This was found to be the case initially when the platelets were incubated with P^{32} for only 3 hours. However this was not the case when the length of incubation was increased to 14 hours. In this instance the radioactivity remained associated with the platelet (Table II).

This information seemed to indicate that the platelets were actively incorporating the phosphorus. Since very little is known about

TABLE II. Demonstration that Prolonged *In vitro* Incubation Firmly Incorporates P^{32} into Platelet as Compared to Shorter Incubation Periods.

Specimen	Counts/min./1 ml — After 3 hr incubation	After 14 hr incubation
1st wash	10260	7440
2nd "	1080	2136
3rd "	720	1950
4th "	636	
5th "	540	
Platelet suspension	11700	21360
Wash water after overnight incubation	10680	2430
Overnight platelet suspension	1020	18930

TABLE III. Demonstration Depicting Kinetics of Assimilation of P^{32} into Rabbit Platelet after Varying Periods of *In vitro* Incubation.

Specimen		Counts/min./1 ml — After 3 hr incubation	After 14 hr incubation
Phosphorous compartment	Acid sol. P.	4140	2580
	Fat sol. P.	120	2460
	Organic P.	72	20460

TABLE IV. Average Recovery of Radioactivity in Blood of 10 Rabbits Following Intravenous Injection of Homologous Radioactive Platelet Suspension.

Sample of blood	% of inj. activity/ml blood	% of inj. activity totally recovered/ml (blood vol, 250 cc approx.)
Immediate (theoretical)	.4	100
1-3 min.	.14	34
2 hr	.01	3

platelet metabolism it was decided to study in more detail the kinetics of assimilation of the P^{32} into the cell. The 3 phosphorous compartments, acid-soluble, lipid and organic, were explored for radioactivity by subjecting the tagged platelets to simple extraction procedures. Eight cc of cold 10% trichloracetic acid added to 4 cc of the platelet suspension removed the acid soluble phosphorus. The precipitate was then treated with a 2:1 hot alcohol-ether mixture which extracted the lipophorphorous components. That which remained was dissolved in 2 N KOH. Aliquots of these extracts were subjected to Geiger counting. The results are given in Table III. After 3 hours incubation with P^{32} most of the radioactivity is in the acid-soluble portion which accounts for the ease with which the P^{32} moved in and out of the platelets. However after 14 hours (overnight) most of the phosphorus is in the organic compartment where it is more tightly bound. From this set of experiments it would appear that the labelling technic herein described is applicable to a study of the life span of transfused rabbit platelets.

Therefore radioactive rabbit platelet suspensions containing 500000 to 1000000 platelets per cmm and representing between 200000 and 700000 beta counts/minute were reinjected into an ear vein of the same animal from which they had previously been isolated. Platelet counts performed on the rabbit's blood before and after the transfusion failed to reveal any consistent change. The radioactivity of the peripheral blood was followed by obtaining 1 cc specimens of heart blood for Geiger counting at varying intervals after the injection. In most instances the interval was 2 hours although some samples were taken at 4 and 24 hours. In the latter cases there was no variation from that obtained at two hours. It is apparent from Table IV that the activity disappeared from the blood rather promptly and in fact was almost completely gone after 2 hours.

At the end of 2 hours the animals were sacrificed, various organs removed *in toto* when possible, and aliquot portions ashed and counted for radioactivity. The recovered activity was found distributed primarily in the organs of high reticulo-endothelial activity,

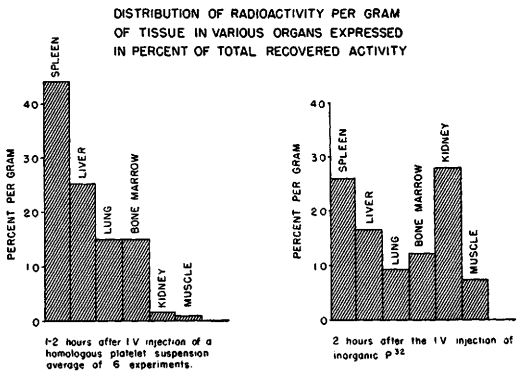

FIG. 2.

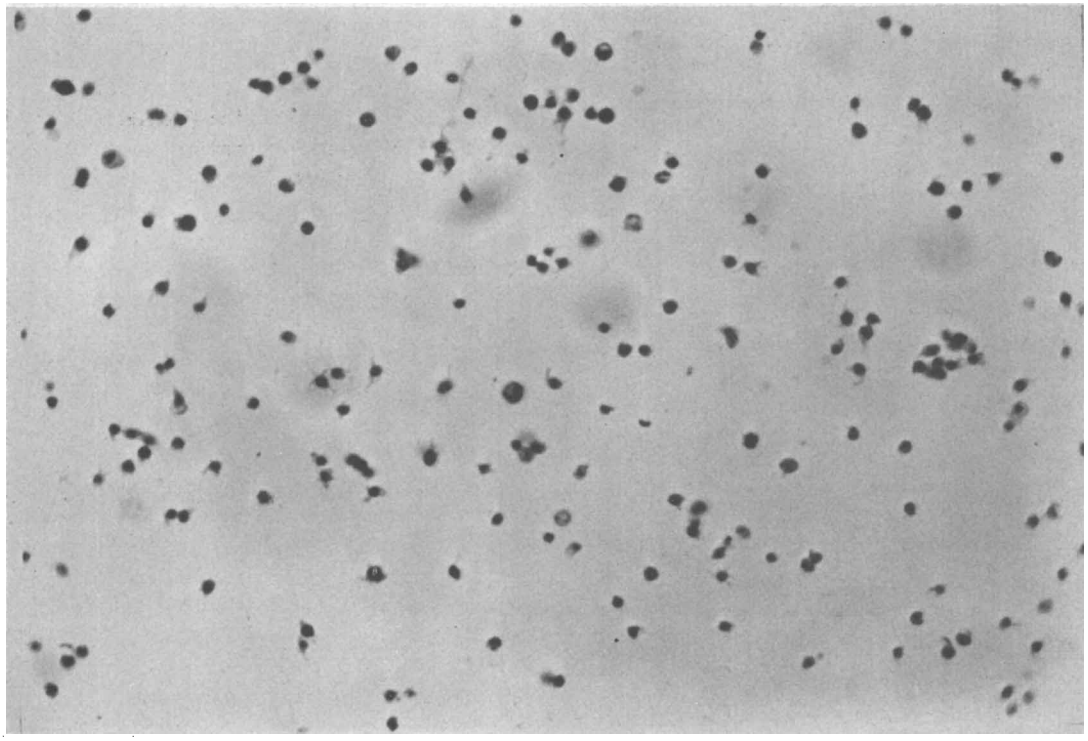

FIG. 3.

the spleen, liver, lung and bone marrow in that order (Fig. 2) when expressed in terms of recovered activity per gram of tissue.

These data would indicate that under the conditions of the experiment transfused radioactive platelets disappear from the circulating blood quite rapidly in the normal rabbit and that the reticulo-endothelial system, in particular the spleen, plays a prominent role in this process.

The following tests were performed on the radioactive platelets to determine viability. 1) The platelets stained with brilliant cresyl blue have normal morphology (Fig. 3). 2) Clots prepared from purified fibrinogen retract upon the addition of radioactive platelets. However these platelets will not restore retraction to a natural clot prepared from platelet-free plasma. 3) The abnormal serum prothrombin time of platelet-free plasma is restored to normal upon the addition of radioactive platelets (Table V). 4) Endogenous respiration as measured in a Warburg does occur, but is greater in the treated than in the non-treated platelets. This observation may indicate that some damage has occurred during manipulation.

Discussion. In recent experiments performed by several investigators, the life span of the human platelets has been estimated to be 3-6 days(3-5). This figure has been derived by the indirect method of following the platelet count of human subjects following the transfusion of platelet rich blood or platelet suspensions into these individuals. Earlier work in animals by Duke(6) had indicated that the life span of platelets is measured in days rather than minutes or hours. The present report does not agree with these findings. The immediate question arises: are these platelets, which admittedly go through a traumatic procedure "normal"? The results of the viability tests reported above indicate

TABLE V. Serum Prothrombin Time.

Material	Time, sec.
Native serum	150
Serum from platelet-free plasma	16
Serum from platelet-free plasma plus radioactive platelets	180

that the radioactive platelets have been damaged to some degree.

It is obvious that more investigation must be done before a final answer can be given but these experiments do indicate the sites where platelets may be destroyed and point up certain aspects of platelet metabolism heretofore not demonstrated. It is hoped in the future that this method may be applied to studies in human beings.

Summary. 1. Purified, unagglutinated platelet suspensions prepared from rabbit blood do incorporate radioactive phosphorus readily, and therefore lend themselves to life span studies *in vivo*. 2. These studies indicate that transfused rabbit radioactive platelets are rapidly removed from the peripheral blood and the radioactivity recovered in organs of the reticulo-endothelial system, namely spleen, liver, lung and bone marrow. 3. The problem of viability of these platelets is discussed. The evidence suggests that damage has occurred during manipulation. 4. These observations are important primarily in demonstrating aspects of metabolism and destruction of platelets.

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Reversible Inhibition of Cell Division and Enlargement in Plant Tissues by 2, 6-Diaminopurine.* (20418)

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Since the report by Hitchings and coworkers(1) that 2,6-diaminopurine (DAP) inhibits the growth of *Lactobacillus casei*, the compound has been used in many studies of growth in microorganisms and animals as well as in virus reproduction. In survey experiments, DeRopp(2) tested the chemical for action on growth of plant tumor tissue but observed no effects. In this paper are reported the results of experiments with several different tissues of higher plants in which DAP inhibits cell division—as it does in animals and microorganisms—and also inhibits cell enlargement and the formation of vegetative buds.

Cell division in tobacco stem segments. Sterile tobacco stem segments were obtained from 3 to 4 ft tall tobacco plants, Wisconsin No. 38, grown in a greenhouse. After removal of the leaves, the stems were cleansed by swabbing with 95% ethyl alcohol and then the outer layers down to the cambium were peeled off. The remaining cylinders were cut into sections 1 cm long. Three to 4 segments were obtained from each section by cutting it tangentially and the central core (pith) was discarded. These stem segments contained parts from the vascular-cambial region, xylem, internal phloem, and pith and averaged about 6 mm in width, 10 mm in length, and 2 mm (maximal) in thickness. They were placed pith side down on the surface of the basal or

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