Intravascular Life Span of the Duck Red Blood Cell as Determined by Radioactive Selenium. (20289)

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Various methods have been employed to estimate the life span of the red blood cell in the human, as well as in experimental animals. The earlier methods in which differential agglutination technics were employed have been reviewed by Isaacs(1). Hawkins and Whipple(2) studied the life cycle of the red blood cell in the normal bile fistula dog by following the bile pigment output after blood destruction or blood withdrawal. Tagging the red blood cell with N^{15}(3), and with P^{32}(4), are the more recent methods used to estimate the life span of the red blood cell. Jope(5) reported that sulfhemoglobin in the blood of T.N.T. workers was found to persist and was still detectable after many weeks. The disappearance of sulfhemoglobin from the blood stream was related to the destruction of the red blood cell which contained it. Further, a linear disappearance of sulfhemoglobin was found which disproved random destruction. In these experiments on man it was estimated that the life span of the red blood cell was 116 days. McKerns and Denstedt(6) have used the disappearance of sulfhemoglobin from the blood as a measure of the intravascular life span of the erythrocyte in rabbits. Dod, Bierman, and Shimkin(7) applied this method in normal rabbits and mice, and in tumor-bearing mice. In a previous publication(8) it was postulated that *in vivo* selenohemoglobin, similar to sulfhemoglobin, could be formed. The fixation of selenium in red blood cells of the rabbit(9) and the dog(10) has been demonstrated, and is shown below in the duck.

In view of the likely possibilities of similar properties of sulfhemoglobin and selenohemoglobin, experiments were carried out to study the rate of destruction of duck red blood cells tagged with radioselenium. The disappearance of selenohemoglobin from the circulation of the duck was therefore studied in detail. It was estimated that the intravascular life span of the duck red blood cell was 11.7 days.

Procedure. Two adult white Pekin ducks weighing 2.0 and 2.5 kg were injected intraperitoneally with sodium selenate containing radioselenium. Each duck received 2.5 x 10^6 c.p.m. radioselenium (8.0 mg selenium) in 3 doses, the second and third doses being administered 16 and 22 hours respectively after the first dose. The ducks were bled from the jugular vein 2 hours after the last injection. The citrated blood of the 2 ducks was pooled, centrifuged, the plasma separated, and the red blood cells washed repeatedly with isotonic saline. The washed, packed red blood cells were stored in the refrigerator until used, then mixed with an approximately equal volume of isotonic saline. This constituted the stock mixture which was administered the same day to recipient ducks. Each of 10 young white Pekin ducks weighing 481-567 g was injected in a leg vein with 10 ml (6 ml packed red blood cells) of the stock mixture which contained red blood cells tagged with radioselenium. Each duck received a total of 1.74 x 10^3 c.p.m. of radioselenium. Two each of the recipient ducks were exsanguinated intracardially as completely as possible after one hour, one day, 3 days, 7 days, and 10 days. The citrated blood was centrifuged, plasma aspirated, and the R.B.C.'s washed repeatedly with isotonic saline. The volume of packed red blood cells for each duck was recorded. The washed red blood cells were then wet ashed with concentrated HNO_3 and 30% H_2O_2, and radioactivity measurements were made similar to those previously outlined(8,11). In a separate experiment crystalline hemin was isolated from the blood of 4 ducks that had been injected with varying amounts of sodium selenate containing radioselenium. The methods used have been described previously(10). It was observed that samples of crystalline hemin contained 72.9, 177.1, 1008.8, and 396.4 c.p.m. per mM.

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TABLE I. Disappearance of Injected Red Blood Cells Tagged with Radioselenium (Selenohemoglobin) from the Blood of Ducks.

Duck No.	Time after injection	Body wt, g	Observed activity, c.p.m./ml R.B.C.	Corrected activity,* c.p.m./ml R.B.C.
702	0	539		
	1 hr	—	73.2	
703	0	567		
	1	—	103.5	
			Avg 88.4	
704	0	539		
	1 day	580	89.8	97
708	0	481		
	1	530	64.5	71
			Avg 77.2	Avg 84
705	0	510		
	3 days	630	61.2	75.9
706	0	510		
	3	630	54.6	67.7
			Avg 57.9	Avg 71.8
709	0	539		
	7	907	18.2	30.6
711	0	510		
	7	794	18.3	28.6
			Avg 18.3	Avg 29.6
710	0	481		
	10	935	7.4	14.3
713	0	481		
	10	964	8.1	16.4
			Avg 7.8	Avg 15.4

* Corrected activities were obtained by multiplying observed activities by a correction factor which was obtained by dividing body wt at time T by body wt at time O (see text).

Results and discussion. In Table I are shown the activities of duck red blood cells at various time intervals after the intravenous injection of red blood cells tagged with radioselenium. The results are expressed as c.p.m. per ml of R.B.C.'s. It was necessary to correct (column 5) the observed activities in order to account for the dilution of the tagged cells produced by the increase with age of total blood volume. The corrected activities were calculated by multiplying the observed activities by a dilution factor which was obtained by dividing the body weight at time T by the weight at time O. Since hematocrits and percentages of body weight as blood remain essentially constant during the experimental period, the ratio of red blood cell volumes is proportional to the weights. It will be noted that the average activity value for 2 ducks at each time T shows a progressive decline with time.

The first blood samples taken at the one-hour interval (Ducks 702 and 703) represent well-mixed and maximum blood activity samples. If the average value 88.4 c.p.m. per ml R.B.C. for the first hour samples is taken as the maximum or initial blood activity level, the percent of original activity for the 1st, 3rd, 7th, and 10th days would be 95.1%, 81.3%, 33.5%, and 17.4%, respectively.

In Fig. 1 is shown a plot of R.B.C. activities (% of initial value) against time in days. The straight line was constructed on the basis of least squares of perpendiculars to the line, giving a line described by: $y = 100-8.5x$, where y = percentage of injected activity, and x = time in days. It will be noted that the line intersects the x axis at 11.7 days. This value of 11.7 days is interpreted to represent

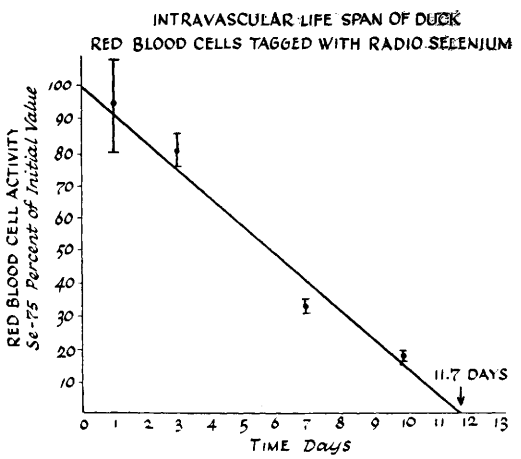

FIG. 1.

the intravascular life span of the duck erythrocytes under experimental conditions described here. The linear disappearance of selenium-tagged red blood cells in the duck indicates, as Jope(5) has shown in the case of sulfhemoglobin in man, that the red cell has a fixed life span. To further clarify the assumption that the x-intercept does in fact represent this mean erythrocyte life, it may be recalled that the donor cells were of all ages but that presumably the mean age of the donor erythrocyte is exactly mid-life. The point of disappearance of activity from the cell presumably represents the destruction of the youngest donor cells. If the Jope interpretation of straight line disappearance is correct, this x-intercept also represents the mean intravascular life of the erythrocyte. Further, the linear disappearance of selenium from the red cell fraction of blood is interpreted to be related to the destruction of red cells, and to show that this process does not take place at random. In correlating the relationship between the disappearance of selenohemoglobin (fixed selenium) and the destruction of the red cells, certain assumptions are made similar to those of Jope(5) on the disappearance of sulfhemoglobin from the blood. It is assumed that the intact red cell does not have the ability of exchanging fixed selenium, and that the organism has no way of removing it other than removal of the red cells which contain selenium. When the cells containing fixed selenium are broken down, the selenium is not incorporated into new red cells. In relating the destruction of the red cells containing selenium to that of normal cells it must be assumed further 1) that the selenohemoglobin formed is distributed initially at random among the red cells of all ages existing in the donor duck at that time, 2) that the formation of selenohemoglobin within the cell does not prolong or curtail its life span, and 3) that the polycythemia induced by donor cells does not materially accelerate destruction.

As far as we have been able to discover, this is the first report on the intravascular life span of the duck R.B.C. At first it appeared that the life span of the R.B.C. in the duck as determined here was very short. However, in another fowl, the hen, Hevesy and Ottesen(4) using P^{32}, and Shemin(12) using N^{15} glycine, have shown the life time of the red nucleated corpuscle to be 28 days, a relatively short time as compared with other species, for example man, which has been estimated to be 127 days(3).

Summary. Use has been made of the ability of selenium to be fixed in red blood cells to estimate the intravascular life span of the duck erythrocyte. Donor ducks were injected with sodium selenate containing radioselenium. These ducks were then bled and the red blood cells injected into 10 young ducks. Two each of the recipient ducks were bled at various time intervals up to 10 days. It was noted that there was a linear disappearance of selenium-tagged red blood cells from the circulation of the ducks. The value of 11.7 days was found to be the intravascular life span of the duck erythrocytes under the experimental conditions described here. In correlating the relationship between the disappearance of fixed erythrocyte selenium and the destruction of the red cell certain assumptions have been made which are discussed.

1. Isaacs, R., *Physiol. Rev.*, 1937, v17, 291.
2. Hawkins, W. B., and Whipple, G. H., *Am. J. Physiol.*, 1938, v122, 418.
3. Shemin, D., and Rittenberg, D., *J. Biol. Chem.*, 1946, v166, 627.
4. Hevesy, G., and Ottesen, J., *Nature*, 1945, v156, 534.
5. Jope, E. M., *British J. Int. Med.*, 1946, v3, 136.
6. McKerns, K. W., and Denstedt, O. F., *Ameri-*

can Red Cross, 1949, Boston, Mass.
7. Dod, K. S., Bierman, H. R., and Shimkin, M. B., *J. Nat. Cancer Inst.,* 1951, v11, 1093.
8. McConnell, K. P., and Martin, R. G., *J. Biol. Chem.,* 1952, v194, 183.
9. McConnell, K. P., Unpublished data.
10. McConnell, K. P., and Cooper, B. J., *J. Biol. Chem.,* 1950, v183, 459.
11. McConnell, K. P., *J. Biol. Chem.,* 1941, v141, 427.
12. Shemin, D., *Cold Spring Harbor Symposia on Quantitative Biology,* 1948, vXIII, 185.

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Effects of Thyroxine, Thyroidectomy and Lowered Environmental Temperature on Incorporation of Deuterium into Cholesterol.* (20290)

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The effects of thyroid- and thiouracil-feeding on the rate of incorporation of heavy hydrogen isotopes into cholesterol were studied in the rat by Karp and Stetten(1), and by Byers and coworkers(2). However, since Fleischmann and coworkers demonstrated recently that α-naphthylthiourea influences cholesterol metabolism not only by way of the thyroid gland, but also by another mechanism, completely independent of the thyroid (3), the interpretation of the above mentioned results presents some difficulties in the case of the hypothyroid groups. It was decided, therefore, to produce a hypothyroid condition in rats by means of thyroidectomy, rather than using anti-thyroid drugs, and to study the rate of incorporation of deuterium into cholesterol in these animals, and, for comparison, in hyperthyroid rats. The results are reported in the present paper.

In the course of these experiments, another difficulty in interpretation arose, *i.e.,* whether the effects observed in the hyperthyroid group were the result of a direct and specific action of the thyroid hormone, or, whether they were solely the consequence of an increase in the metabolic rate in general. Attempts were made to obtain information on this question by means of using animals in which the metabolic rate was increased independently of the thyroid gland; this was accomplished by exposing thyroidectomized rats to a lower environmental temperature in accord with observations reported by Ring(4), and Sellers and You(5). The present report includes results of experiments in which the rate of incorporation of deuterium into cholesterol was compared in thyroidectomized rats kept at 2-5°C, and at 25-30°C.

Methods. Female weanling rats, USC strain, were fed *ad libitum* a diet low in cholesterol.‡ Littermates were used as far as possible. In the first experiment which dealt with the effects of modifications of the thyroid activity, 3 groups of rats were used: One group was injected intraperitoneally with .05 mg thyroxine daily for a period of 3 weeks. Another group was thyroidectomized at an age of 4 weeks, and maintained for 2 weeks to deplete circulating thyroid hormone. Untreated animals of the same age served as

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‡ Composition of diet: 33.8% oats; 33.7% whole wheat; 15.0% non-fat milk powder; 4.5% alfalfa; 2.0% yeast (strain G, Anheuser-Busch); 8.0% cottonseed oil; 2.0% fortified oil (containing 312 U.S.P. units vitamin A and 50 U.S.P. units vit. D per g); 0.5% calcium carbonate: 0.5% sodium chloride.