

THE TURNOVER RATE OF PHOSPHOLIPIDS IN THE PLASMA OF THE DOG AS MEASURED WITH RADIOACTIVE PHOSPHORUS

By D. B. ZILVERSMIT, C. ENTENMAN, M. C. FISHLER, AND I. L. CHAIKOFF

(From the Division of Physiology, University of California Medical School, Berkeley)

(Received for publication, August 24, 1942)

From the considerations presented in the previous paper (1), it is apparent that an exact measure of the turnover of a compound in an organ like kidney, liver, etc. requires knowledge of the "specific activity-time" relations of (1) the compound itself and (2) its precursor. For the determination of the turnover rate of phospholipid phosphorus of plasma, however, the procedure can be greatly simplified, since it is feasible to introduce a small amount of plasma containing the labeled compound and measure its disappearance from the circulating fluid. This procedure makes unnecessary the measurement of the specific activity of the immediate precursor, and so the path of formation of the compound need not be known. The compound, in its labeled form, can be either obtained by synthesis *in vitro* or used as present in plasma removed from an animal that has synthesized the labeled compound. The latter procedure has the advantage in that the labeled phospholipid introduced is of the same type as that present in plasma and its physicochemical state is not altered by chemical treatment. In other words, the isotopic phospholipid whose disappearance is being measured is chemically indistinguishable from the animal's own plasma phospholipid. The above principles have been applied in the present investigation to determine the rate at which phospholipid phosphorus is turned over in the plasma of the dog.

EXPERIMENTAL

Dogs 1A and 2A served as donors. They received intraperitoneal injections of 2 to 3 millicuries of labeled inorganic phosphate. 26 hours later, blood was removed from the dogs, heparinized, and plasma separated by centrifugation.

The turnover of plasma phospholipid was determined in dogs 1B, 2B, and 3B. The following amounts of blood were removed from these dogs: 50 cc. from dog 1B, 20 cc. from dog 2B, and 20 cc. from dog 3B. Immediately thereafter 100 cc. of radioactive plasma obtained from dog 1A was injected into the femoral vein of dog 1B, whereas dogs 2B and 3B were injected with 70 cc. of radioactive plasma obtained from dog 2A. During the next 5 hours eight blood samples were removed from dogs 1B, 2B, and 3B for the determination of total phospholipid, total P³², and phospholipid P³². During this time, a total of 50 cc. of blood was removed from dogs 1B, 2B, and 3B. After removal of the last blood sample, the three dogs were sacrificed by means of an intravenous injection of nembital. Liver, kidney, small intestine, muscle, and spleen

were removed and their content of phospholipid P^{32} and total phospholipid phosphorus determined. These constituents were also determined in corpuscles that were washed twice with Ringer's solution.

For the determination of total P^{32} in plasma, 1 cc. of the latter was pipetted into a volumetric flask and aliquots mounted on blotters for the determination of its radioactivity, in a manner described elsewhere (2).

TABLE I
Radioactive Phosphorus Content of Plasma

All values of P^{32} expressed as counts per minute per cc.

Min. after plasma injection	TCA* precipitate	Petroleum ether extract	Total	Min. after plasma injection	TCA* precipitate	Petroleum ether extract	Total	Min. after plasma injection	TCA* precipitate	Petroleum ether extract	Total
Donor† Dog 1A (8.5 kg.)				Donor‡ Dog 2A (6.6 kg.)				Donor§ Dog 2A (6.6 kg.)			
	25,400	25,350	31,500		21,600	21,040	30,400		21,600	21,040	30,400
Recipient Dog 1B (8.8 kg.)				Recipient Dog 2B (6.6 kg.)				Recipient Dog 3B (5.7 kg.)			
11	4,890			10	4,850		4,925	12	4,540		5,140
24	4,525		4,710	25	4,320		4,400	28	4,235		4,270
46	4,330	4,630		52	4,220		4,250	47	3,970	3,890	
82	4,055		4,150	92	3,660		3,695	88	3,435		3,675
115	3,890			120	3,395		3,145	117	3,390		3,260
175	3,330		3,840	180	2,905		2,875	177	2,850		2,780
235	3,090	2,890	2,910	240	2,620	2,720	2,660	237	2,640		2,655
300	2,750	2,800	2,770	300	2,340	2,335	2,450	297	2,300	2,360	2,340

* Trichloroacetic acid. See text.

† Plasma was taken from donors 26 hours after the injection of P^{32} . Recipient dogs were in the postabsorptive state at the time when radioactive plasma was injected.

§ Dogs 2B and 3B received 70 cc. of plasma obtained from dog 2A; dogs 2B and 3B thus received a total of 1,472,800 counts of phospholipid P^{32} .

|| Dog 1B received 100 cc. of plasma obtained from dog 1A; dog 1B thus received a total of 2,540,000 counts of phospholipid P^{32} .

Phospholipids were isolated from tissues and plasma by extraction with an alcohol-ether mixture (3:1). The extracts were concentrated to a low volume and phospholipids taken up with petroleum ether. For the determination of phospholipid P^{32} , aliquots of the petroleum ether extract were mounted on blotters and their radioactivity determined with the Geiger counter. Phospholipid phosphorus was determined in the same petroleum ether extract. The color, developed according to King's method (3), was measured with a photoelectric colorimeter.

P^{32} content of the trichloroacetic acid-insoluble fraction of plasma was determined

as follows: 1 cc. of plasma was transferred dropwise to a centrifuge tube containing 10 cc. of 10 per cent trichloroacetic acid and the mixture vigorously agitated with a glass rod. It was allowed to stand for 10 minutes, during which it was thoroughly agitated several times to bring the precipitate to a finely suspended state. The mixture was then centrifuged for 5 minutes, the supernatant fluid decanted, the precipitate washed once with 5 cc. of 10 per cent trichloroacetic acid, and the suspension again centrifuged. The precipitate was dissolved in a few drops of 10 per cent NaOH and quantitatively transferred to a volumetric flask; aliquots were then taken for the determination of P^{32} .

The radioactive units expressed as counts per minute for (1) P^{32} content of the trichloroacetic acid-insoluble fraction, (2) the phospholipid P^{32} , and (3) total P^{32} for plasma of donor and recipient are recorded in Table I.

Table I shows a striking agreement between the values for the P^{32} content of the trichloroacetic acid precipitate obtained from plasma and the P^{32} content of the petroleum ether extract prepared from plasma. In order to avoid the removal of excessive amounts of blood from dogs 1B, 2B, and 3B, the P^{32} content of the trichloroacetic acid precipitate of plasma was taken as equivalent to its phospholipid P^{32} content.

RESULTS

In the preceding paper the following equation was derived for the disappearance of phospholipid phosphorus from the circulating fluid:

$$\frac{x}{r} = ce^{-\frac{p}{r}t} \quad (1)$$

or

$$\frac{x}{f} = \left(\frac{r}{f}\right) ce^{-\frac{p}{r}t} \quad (2)$$

in which

- p = rate of disappearance of phospholipid phosphorus from the circulating fluid (this is assumed to be constant),
- x = the amount of phospholipid P^{32} (counts per minute) present in the circulating fluid at any time,
- r = the total amount of phospholipid phosphorus (mg.) present in the circulating fluid (this is also assumed to be constant),
- f = the amount (cc.) of circulating fluid in the organism.

Therefore, $\frac{x}{f}$ gives the counts of phospholipid P^{32} per cc. of plasma at any time, and $\frac{r}{p}$ is equal to the turnover time of phospholipid phosphorus in the

circulating fluid, while $\frac{r}{f}$ is a measure of the phospholipid phosphorus level $\left(\frac{\text{mg.}}{\text{cc.}}\right)$ in the circulating fluid.

Since

$$\log \frac{x}{f} = \log \left(c \frac{r}{f} \right) - \frac{p}{r} t \log e, \quad (3)$$

the slope of the straight line (Fig. 1) obtained by plotting the log of the P^{32} values for the trichloroacetic acid precipitate per cc. of plasma (*i.e.* $\log \frac{x}{f}$) against time (t = minutes after the injection of plasma) is $\frac{p}{r} \log e$.

TABLE II
Values for f , t_i and p in Plasma

Dog.....		1B	2B	3B
Plasma volume (f).....	Cc.....	535	323	339
	Per cent of body weight...	6.6	4.9	6.0
Turnover time (t_i), hrs.....		9.3	7.2	7.8
Turnover rate (p), mg. phospholipid phosphorus.....		7.0	5.2	8.0

Turnover Time.—Turnover time for phospholipid phosphorus can be determined from Fig. 1. Thus

$$t_i = \frac{r}{p} \text{ and from equation (3) the slope of the curve is } -\frac{p}{r} \log e; \text{ therefore}$$

$$t_i = -\frac{\log e}{\text{Slope}} = -\frac{0.434}{\text{Slope}}$$

The values for turnover time for phospholipid phosphorus of dogs 1B, 2B, and 3B are shown in Table II.

Turnover Rate.—To determine the turnover rate of phospholipid phosphorus in the circulating fluid it is necessary to know t_i and the total amount of phospholipid phosphorus present in the circulating fluid. The latter can be obtained from two measurements; (1) the concentration of the phospholipid phosphorus in the circulating fluid (mg. per cc.), and (2) the volume of the circulating fluid of the dog. The amount of the circulating fluid for each dog can be determined as follows:

The slopes of the curves in Fig. 1 are constant after the 20 minute interval

and smaller than the slopes between 0 and 20 minutes. The greater slopes found during the early intervals may be explained by supposing a non-uniform distribution of the injected radiophospholipid in the circulating fluid during

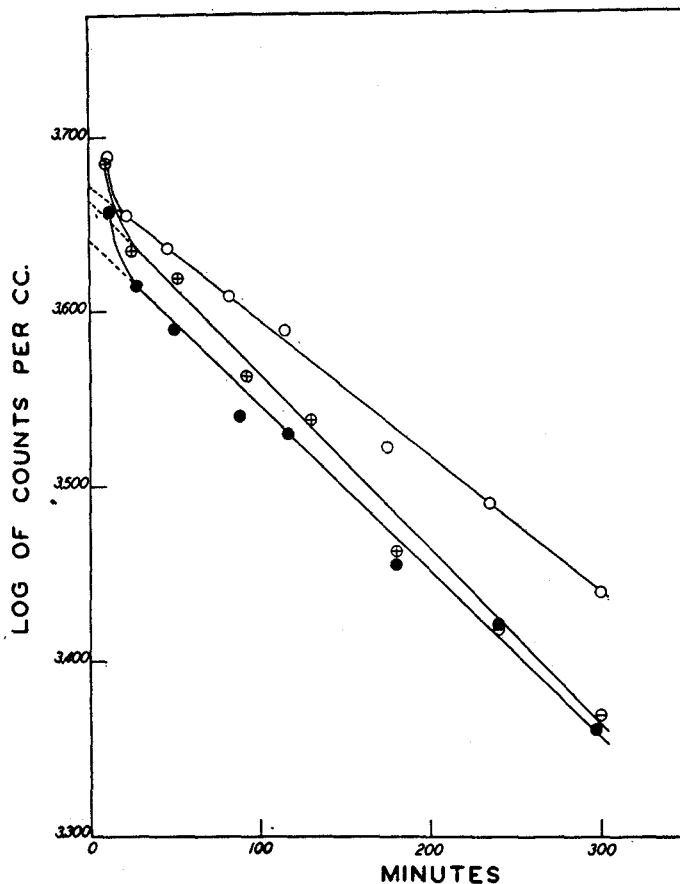


FIG. 1. The logarithm of the counts per cc. of plasma phospholipid phosphorus as plotted against time. \circ = Dog 1B, \oplus = Dog 2B, \bullet = Dog 3B.

this interval. There is no reason to doubt that a uniform distribution of the injected phospholipid phosphorus had occurred at the later intervals. For this reason one is justified in extrapolating the straight-line curves to "zero-time" in order to obtain the number of counts per cc. of phospholipid P^{32} that would have been present in the circulating fluid if the specific activity of the phospholipid phosphorus through all parts of the circulating fluid had been the same at "zero-time." Mathematically, this corresponds to determining

$\log \left(c \frac{r}{f} \right)$. When $t = 0$, $\log \left(c \frac{r}{f} \right) = \log \frac{x}{f}$. Therefore by dividing the number of counts of phospholipid P^{32} per cc. of plasma at "zero-time" $\left[\left(\frac{x}{f} \right)_{t=0} \right]$ into the total amount of phospholipid P^{32} injected, the volume in which the phospholipid P^{32} has distributed itself is obtained.

The values for the volume of circulating fluid (f) are shown in Table II. These values agree with plasma values obtained in dogs by the dye-injection methods (4). So it is reasonable to assume that the injected phospholipid is *initially mixed only with plasma* of the recipient dogs and that the penetration of injected phospholipid into other tissues is a relatively slow process.

The turnover rate of phospholipid phosphorus (p) can now be obtained from the total amount of phospholipid phosphorus in the plasma (r) and its turnover time (t_t) as follows:

$$p = \frac{r}{t_t}$$

The values for p are recorded in Table II.

DISCUSSION

Previous studies of the fate of injected labeled phospholipid were made by Hahn and Hevesy (5) and by Haven and Bale (6). The latter injected emulsions of labeled phospholipid extracted from rat tissues and determined its disposition in the tissues of the rat. Hahn and Hevesy, however, injected plasma containing labeled phospholipid. Although compounds other than phospholipid are injected into the animal when plasma is employed, it has been shown in this laboratory that these cause no appreciable increase in radioactive phospholipid synthesis in the liver in the interval studied. Thus 2,540,000 radioactive units were injected into dog 1B; of this amount 600,000 were present in compounds other than phospholipid. It has been shown that in 5 hours 0.01 per cent of injected inorganic P^{32} is converted to phospholipid per gm. of liver in the dog (7). Of the 14,400 counts of phospholipid P^{32} found per gm. of liver in dog 1B, 60 counts were probably synthesized from phosphate injected in forms other than phospholipid.

The distribution of phospholipid P^{32} in the tissues of the three dogs 5 hours after the injection of the labeled plasma is shown in Table III. A large fraction (51-58¹ per cent of that injected) of the phospholipid P^{32} is still present

¹ These values are sufficient to give the turnover time of plasma phospholipid phosphorus once it has been established that the curves in Fig. 1 are straight lines. Thus by substitution in equation (1)

$$0.58 = 1 \cdot e^{-5/t_t}, \quad t_t = 9.1 \text{ for dog 1B;}$$

$$0.51 = 1 \cdot e^{-5/t_t}, \quad t_t = 7.5 \text{ for dog 2B;}$$

$$0.53 = 1 \cdot e^{-5/t_t}, \quad t_t = 7.8 \text{ for dog 3B;}$$

in the plasma at the end of 5 hours. At complete turnover time (*i.e.* after 9 hours for dog 1B, after 7 hours for dog 2B, and after 8 hours for dog 3B) 37 per cent of the injected phospholipid P³² would still be present in the plasma.

TABLE III
Distribution of Phospholipid (PL) in Tissues at the End of 5 Hours

1	Dog 1B			Dog 2B			Dog 3B		
	2	3	4	5	6	7	8	9	10
	Per cent of Injected dose PLP ³² per whole organ after 5 hrs.	Amount of PLP per whole organ*	Per cent of Organ's PL supplied by plasma PL per hr. †	Per cent of Injected dose PLP ³² per whole organ after 5 hrs.	Amount of PLP per whole organ	Per cent of Organ's PL supplied by plasma PL per hr. †	Per cent of Injected dose PLP ³² per whole organ after 5 hrs.	Amount of PLP per whole organ	Per cent of Organ's PL supplied by plasma PL per hr. †
		mg.			mg.			mg.	
Plasma.....	57.7	12.2§	—	51.4	11.6§	—	53.0	18.5§	—
Liver.....	16.2	255	1.08	15.2	169	0.93	11.1	173	1.09
Kidney.....	0.76	31.6	0.41	1.27	24	0.55	1.13	28.6	0.67
Small intestine.....	—	—	—	2.59	54	0.50	2.44	68	0.61
Spleen.....	—	—	—	0.30	8.2	0.38	0.35	8.5	0.71
Cells 	0.36	—	—	0.35	—	—	1.15	—	—
Muscle.....	7.4**	—	—	4.7**	—	—	4.2**	—	—

* PLP refers to phospholipid phosphorus.

† Breakdown of phospholipid P³² is disregarded.

§ Mg. phospholipid phosphorus per 100 cc.

|| Washed twice with Ringer's solution.

** Analysis of gastrocnemii muscles was made. Values for whole organ based on muscle constituting 40 per cent of body weight.

This can be shown as follows: By substitution of $t = \frac{r}{p}$ in equation (1),

$$\frac{x}{r} = ce^{-1} = 0.37c,$$

where c = the specific activity of the plasma phospholipid phosphorus at "zero-time."

Next to plasma, the liver contained the largest amounts of phospholipid P³². These findings are in agreement with those reported by Hahn and Hevesy (5), and by Haven and Bale (6).

Information about the transport of plasma phospholipid to the different organs can be obtained from the data presented here. In columns 4, 7, and 10 of Table III is shown the percentage of each organ's phospholipid that is

supplied per hour by the plasma.² In the case of the liver, 1 per cent of its phospholipid is obtained directly from plasma phospholipid per hour. In the kidney and small intestine, about 0.5 per cent is so derived. In these calculations we have chosen to neglect loss of the phospholipid P³² in the organs examined since no reliable data on breakdown or leaving of phospholipid are available. The data in Table III (columns 2, 5, and 8) show that as much as 76–83 per cent of the injected phospholipid P³² can be accounted for by the seven tissues examined in the present investigation. These values would appear to exclude a rapid breakdown of phospholipid in the animal.

SUMMARY

1. A method for the determination of turnover time and turnover rate of plasma phospholipid is presented.
2. During the postabsorptive state 5.2 to 8.0 mg. of phospholipid phosphorus are turned over per hour in the plasma of dogs weighing 6–9 kilos.
3. The amount of phospholipid in an organ that is supplied by plasma phospholipid per hour is calculated.

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² Let "q" be the rate of transport of plasma phospholipid to an organ and "a" be the number of counts of phospholipid P³² present in an organ at time t. Since the specific activity of plasma phospholipid phosphorus equals

$$\frac{x}{r} = ce^{-\frac{p}{r}t}, \quad a = \int_0^t qce^{-\frac{p}{r}t} dt$$

by integration

$$q = \frac{a}{c \left[\frac{e^{-\frac{p}{r}t} - 1}{-\frac{p}{r}} \right]} = \frac{a}{c \left[1 - e^{-\frac{p}{r}t} \right] t}$$

For the liver of dog 1B

$$q = \frac{412,000}{\frac{4710}{0.122} \left[1 - e^{-\frac{5}{9.34}} \right] 9.34} = 2.76$$

i.e., 2.76 mg. of phospholipid phosphorus enters the liver of dog 1B per hour. Hence the percentage of the liver phospholipid supplied by plasma phospholipid = $\frac{2.76 \times 100}{255} = 1.08$ per cent. Hevesy and Hahn (8) used a similar approach to calcu-

late the percentage of plasma phospholipid transferred from plasma to liver.