mentation rate of erythrocytes or the agglutination of Group A hemolytic streptococci. It is probably unrelated to the conglutinin reaction of Bordet and Gay[5] since complement is not required for its effect. The precise nature of the substance and its significance with regard to the rheumatoid state remain to be determined. In any event, the present observations indicate that the sheep cell differential agglutination test may be of value in determining the activity status of patients suffering from rheumatoid arthritis. It may also prove to be helpful as an aid in the differential diagnosis between rheumatoid arthritis and other diseases with arthritic manifestations, such as rheumatic fever.

Summary. A differential sheep cell agglutination test is described wherein the agglutination titers of human serum for normal and sensitized sheep erythrocytes are compared. High differential titers have been found to occur almost solely with the sera of patients suffering from active rheumatoid arthritis. Some of the features of this serological phenomenon are pointed out, together with its possible practical applications.

[5] Bordet, J., and Gay, F. P., *Ann. Inst. Pasteur*, 1906, **20**, 467.

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Phospholipid Metabolism in Diabetes: Turnover Rate of Plasma Phospholipids in Completely Depancreatized Dogs.

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The increased amounts of fatty acids that appear in both liver and plasma of dogs deprived of all islet tissue indicate that diabetes in this animal is characterized by a severe disturbance in fat metabolism.[1,2] It is shown here, however, that the turnover of plasma phospholipids is not altered in the diabetic dog. Apparently, plasma phospholipids do not participate in the redistribution of fat that occurs in the diabetic dog.

Experimental. Depancreatized Dogs. After complete excision of the pancreas the dogs were injected with insulin and fed twice daily a mixture containing lean meat, raw pancreas, sucrose, and vitamin supplements.[3]

Synthesis of Labeled Plasma Phospholipids. To provide labeled plasma phospholipids, 2-3 millicuries of radioactive phosphate were administered by stomach tube to fasted normal dogs. Twenty-four hours later the dogs were bled, the heparinized blood centrifuged, and the plasma containing the radioactive phospholipid separated.

Methods of Analyses. Phospholipid P^{31} and phospholipid P^{32} were determined in plasma in a manner described elsewhere.[4] The method used for determination of plasma total-fatty acids has also been recorded elsewhere.[5] At the end of the period of observation, the whole liver was excised, thoroughly ground, and analysed for its total-fatty acid content.[6]

Experiment 1. 50 cc of plasma containing labeled phospholipids were injected intravenously into a normal and a depancreatized dog, the latter of which had been deprived of insulin but not food for 3 days. Samples of blood were removed from both dogs at several intervals and the plasma analysed for

* Fellow of the American Cancer Society (recommended by the Growth Committee).

[1] Gibbs, G. E., and Chaikoff, I. L., *Endocrinology*, 1941, **29**, 885.

[2] Bloor, W. R., Gillette, E. M., and James, M. S., *J. Biol. Chem.*, 1927, **75**, 6.

[3] Montgomery, M. L., Entenman, C., and Chaikoff, I. L., *Am. J. Physiol.*, 1944, **141**, 216.

[4] Zilversmit, D. B., Entenman, C., Fishler, M. C., and Chaikoff, I. L., *J. Gen. Physiol.*, 1943, **26**, 333.

[5] Chaikoff, I. L., and Kaplan, A., *J. Biol. Chem.*, 1934, **106**, 267.

[6] Kaplan, A., and Chaikoff, I. L., *J. Biol. Chem.*, 1935, **108**, 201.

TABLE I.
Turnover of Plasma Phospholipids in a Normal and a Diabetic Dog.

Dog		Body wt, kg	Plasma phospholipid turnover time, hr	Plasma phospholipid turnover rate, mg phosphorus per hr
P-70	Normal	10	10.8	6.9
R-70	Depancreatized without insulin	6.2	8.6	7.9

Injected plasma contained 5,800 r.u. phospholipid P^{32} per cc and 2,480 r.u. acid-soluble P^{32} per cc.

At the end of the period of observation, the plasma of the depancreatized dog contained 586 mg % of total fatty acids and 12 mg % phospholipid phosphorus. Its liver contained 12% fatty acids.

total-fatty acids, phospholipid P^{31}, and phospholipid P^{32}. The results are recorded in Table I. In Fig. 1 a semi-log plot has been made for the values of plasma phospholipid P^{32} against time.

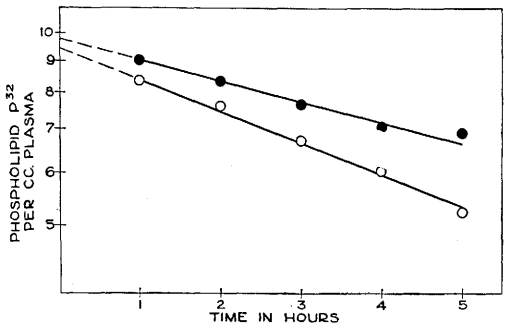

FIG. 1.

The disappearance of intravenously injected labeled phospholipids from the circulation (semi-log plot).

● Normal dog.

○ Depancreatized dog R-70.

In a previous communication[4] it was shown

$$\frac{x}{f} = \frac{cr}{f} e^{-\frac{p}{r}t} \qquad (A)$$

in which x = amount of phospholipid P^{32} present in the entire circulating fluid

f = amount of circulating fluid

c = a constant

r = total amount of phospholipid phosphorus present in the entire circulating fluid

p = rate of phospholipid phosphorus turnover

t = time after injection of phospholipid P^{32}.

Since the turnover time† (t_t) equals $\frac{r}{p}$ we can also write

$$\frac{x}{f} = \frac{cr}{f} e^{-\frac{t}{t_t}} \qquad (B)$$

$$\text{or} \quad \ln \frac{x}{f} = \ln \frac{cr}{f} - \frac{t}{t_t} . \qquad (C)$$

The turnover time (t_t) may conveniently be obtained from Fig. 1 or 2 as follows: At half-time ($t_{1/2}$) the phospholipid P^{32} per cc plasma ($x_{1/2}$) is exactly half that at zero time (t_o). Hence from equation C it follows that

$$\ln \frac{x_o}{x_{\frac{1}{2}}} = \ln 2 = \frac{t_{\frac{1}{2}}}{t_t} \qquad (D)$$

$$\text{or} \quad t_t = 1.44\, t_{\frac{1}{2}} . \qquad (E)$$

The half time can be read from the curves in Fig. 1 and 2 and the turnover time calculated by means of equation E. The turnover rate can next be calculated from the relation:

$$t_t = \frac{r}{p} \qquad (F)$$

The total amount of phospholipid P^{31} present in the entire circulating fluid, *i.e.*, r, can be found if the circulating volume (f) and the concentration of phospholipid P^{31} in plasma ($\frac{r}{f}$) are known. At "zero time" the amount of phospholipid P^{32} present in the circulation ($= x_o$) is equal to the amount of phospholipid P^{32} injected. The circulating volume can be found by dividing the amount of phospholipid P^{32} injected by the concentration of phospholipid P^{32} in plasma at zero

† Turnover time of plasma phospholipids is the time required for the formation of an amount of phospholipid equal to that present in plasma.

TABLE II.
Turnover of Plasma Phospholipids in Depancreatized Dogs Maintained With and Without Insulin.

Dog	Body wt, kg	With insulin*				Without insulin†				
		Plasma phospholipid P31, mg %	Plasma total fatty acids, mg %	Phospholipid turnover time, hr	Phospholipid turnover rate, mg phosphorus per hr	Plasma phospholipid P31, mg %	Plasma total fatty acids, mg %	Liver total fatty acids, % wet wt	Phospholipid turnover time, hr	Phospholipid turnover rate, mg phosphorus per hr
A	5.8	23	737	6.9	12	21	749	12.6	15.1	5.2
B	9.8	16	480	8.5	15	19	894	16.0	10.8	12
C	13.0	18	369	6.3	21	29	958	13.1	9.4	18

* Injected plasma contained 4,520 r.u. phospholipid P32 per cc and 2,560 r.u. acid-soluble P32 per cc.
† Injected plasma contained 13,700 r.u. phospholipid P32 per cc and 10,200 r.u. acid-soluble P32 per cc.

time ($\frac{X_o}{f}$). The value for $\frac{X_o}{f}$ can be read from the intersection of the extrapolated curve with the "Y" axis.

From Table I it is apparent that the turnover rate of plasma phospholipid in the diabetic dog did not differ much from that of the normal. The values obtained for the normal dog are in good agreement with those reported earlier.[4]

Experiment 2. In the next experiment the turnover of plasma phospholipid was compared in the *same* depancreatized dog (1) while it was under insulin control and (2) 4 days after insulin injections had been discontinued. The results are recorded in Table II and Fig. 2.

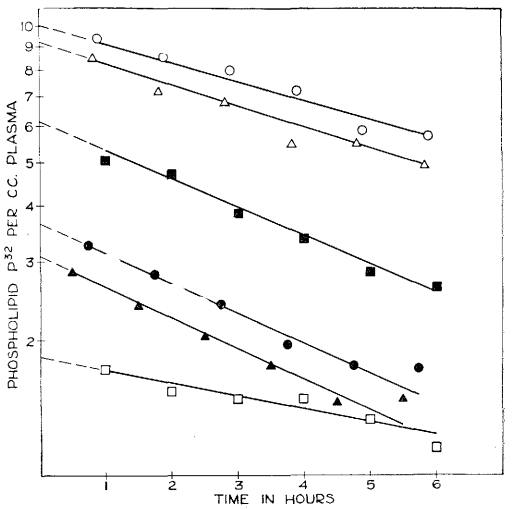

FIG. 2.
The disappearance of intravenously injected labeled phospholipids from the circulation (semi-log plot).

Dog	A	B	C
With insulin	■	●	▲
Without insulin	□	○	△

Discussion. The discontinuation of insulin administrations in the 4 depancreatized dogs resulted in an accumulation of 12-16% fatty acids in their livers (Table II). A transfer of fat from depot to liver has been suggested as the mechanism by which this type of fatty liver develops.[7] It can be calculated that an average of approximately 400 mg of fatty acids were deposited per hour in these livers. If phospholipids serve as vehicles for carrying this extra amount of fatty acids to the

[7] Best, C. H., *Am. J. Digest. Dis.*, 1946, **13**, 155.

liver, then the normal turnover of plasma phospholipid phosphorus would have been increased by an additional $\frac{400}{520} \times 31$ or 24 mg per hour.‡ The results obtained here clearly demonstrate that such an increase in the turnover of plasma phospholipid P did not occur. Hence, it would appear that plasma phospholipids do not participate in the disturbance in fatty acid metabolism encountered in the diabetic state.

The view that phospholipids are concerned with fatty acid transport is widely held today. Such a function for phospholipid, however, has received no support from several types of studies carried out recently in this laboratory:

In the diabetic dog there is reason to believe that considerable amounts of fat migrate from depot to liver. Yet, as noted above, the rate of turnover of plasma phospholipid in this condition was no greater than normal.

In dogs in which the liver is excised the concentration of plasma phospholipids remains constant, and their turnover practically stops.[8] This finding and other evidence show not only that plasma phospholipids are synthesized in the liver, but that this organ is the only one concerned with their utilization. These studies thus fail to support the concept that fatty acids are transported between liver and other organs via phospholipids.

Recent unpublished observations dealing with the mechanism of action of choline[9] are also pertinent here. It was shown that choline does not affect the turnover rate of plasma phospholipids, but that it does stimulate the turnover of lecithin within the liver itself. This seems to indicate that phospholipids, while mediating the lipotropic action of choline, do not bring this about by increasing the transport of fatty acids.

Finally, mention should be made of the studies on the turnover rate of the small intestine's phospholipids in the fasted and fat-fed dog and rat.[10] Here again it appeared that phospholipids are not instrumental in the transport of fatty acids across the intestinal wall.

Summary. The rate of turnover of plasma phospholipids is not increased above normal in the diabetic state. This and other evidence reviewed here fails to support the concept that phospholipids are agents for the transport of fatty acids.

‡ In the phospholipid molecule 520 mg of fatty acids are associated with 31 mg of phosphorus.

8 Entenman, C., Chaikoff, I. L., and Zilversmit, D. B., *J. Biol. Chem.*, 1946, **166**, 15.

9 Zilversmit, D. B., Entenman, C., and Chaikoff, I. L., in press.

10 Zilversmit, D. B., Chaikoff, I. L., and Entenman, C., *J. Biol. Chem.*, 1948, **172**, 637.

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Administration of Chloromycetin to Normal Human Subjects.

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The need for information on the results of administration of Chloromycetin to normal human beings became evident shortly after the chemotherapeutic properties of this antibiotic were first noted;[1,2] this became imperative recently when an opportunity presented itself for a field trial of the drug in the treatment of patients with typhus fever.[3]

1 Smadel, J. E., and Jackson, E. B., *Science*, 1947, **106**, 418.

2 Ehrlich, J., Bartz, Q. R., Smith, R. M., Joslyn, D. A., and Burkholder, P. R., *Science*, 1947, **106**, 417.

3 Smadel, J. E., León, A. P., Ley, H. L., Jr., and Varela, G., PROC. SOC. EXP. BIOL. AND MED., 1948, **68**, 12.