0.5% methylene blue to serve as an indicator. The use of an indicator is optional. After being cooled to 46°C the mixture was readjusted, if necessary, to pH 6.0 using the Beckman pH meter. The solution was then brought back to 950 ml with distilled water, tubed in 9.5 ml volumes, autoclaved at 15 lb pressure for 15 minutes, and allowed to cool. To render the medium complete 0.5 ml of sterile, undiluted human serum was added to each tube giving a final volume of 10 ml.

Populations were counted through 11 serial cultures and compared with those in the original medium of Sprince and Kupferberg. It was found that the simplified medium supported populations equal to those in the original medium. Over 50 serial transfers have been maintained to date without visible differences in shape, size and degree of motility of the protozoa. Examination of living specimens by phase microscopy and stain techniques failed to show any difference in morphology. Three strains of *T. foetus*† and one strain of *T. gallinae* have been carried successfully in this medium at this laboratory. The former species requires adjustment of the medium to pH 7. It has recently been observed that all 3 species are satisfactorily supported by Baltimore Biological Laboratory Thioglycollate medium with dextrose and Eh indicator. This medium contained phytone and trypticase. It requires adjustment to pH 6 for *T. vaginalis* and *T. gallinae*. All 3 species require the addition of serum.

Conclusions. 1. Pantothenic acid has been demonstrated to be an essential metabolite for *Trichomonas vaginalis*. 2. A requirement for phosphate has been established. 3. A culture medium containing a greatly reduced number of components has been developed for routine use.

† Received from Dr. B. B. Morgan, University of Wisconsin.

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Turnover Rate of Phospholipid Phosphorus in the Liver of the White Rat.

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The purpose of the experiments reported here is to provide a quantitative estimate of the turnover rate of phospholipid phosphorus in the liver of the white rat under normal conditions of equilibrium, that is, while the actual concentration of the phospholipid phosphorus in the liver is not changing.

The concept of "turnover" is one of dynamic equilibrium in which, during an interval of time *dt*, a number of phosphorus atoms of mass *dm'* "enter" the hepatic phospholipid molecules, and the same number "leave," so that the net mass of phospholipid phosphorus remains unchanged. The rates concerned are defined as follows:

$$r = \frac{dm'}{dt} \qquad (1)$$

$$R = \frac{r}{m} = \frac{dm'}{mdt} \qquad (2)$$

where r is the mass turnover rate; R is the proportional turnover rate; dm' is the mass of phospholipid P which is "turned over" in time dt; m is the mass of phospholipid phosphorus.

The method used for calculation of the rates is based on the formulation of Zilversmit, Entenman and Fishler.[1] These authors

[1] Zilversmit, D. B., Entenman, C., and Fishler, M. C., *J. Gen. Physiol.*, 1943, **26**, 325.

analyzed mathematically the changes of specific activity of a substance which is in equilibrium with a single precursor, the activity of which itself may be changing. Their analysis showed that the proportional turnover rate is equal to the rate of change of specific activity of substance at any instant, divided by the difference of the specific activity of precursor and substance at that instant. If one considers the changes occurring over a finite interval of time, instead of the instantaneous relations, the proportional turnover rate is equal to the change per unit time of activity of substance, divided by the difference of the *mean* specific activity of precursor and substance in the interval. So far as we know, this method has not previously been applied for the calculation of any actual turnover rate.

Changes of radioactivity of hepatic phospholipid have been studied in terms of relative activity, that is the ratio of specific activity of phospholipid phosphorus to that of hepatic or plasma inorganic phosphorus, or in relation to the activity of the phosphate administered.[2-4] These indexes, however, are not measures of the true proportional turnover rate, and will appear as changing values during the course of an experiment, or will vary according to the regimen of administration of radioactive material, even when the turnover rate itself is actually constant.

Hevesy and Hahn,[2] and Hahn and Tyren[5] and Hevesy[6] have suggested calculating the percentage renewal of phosphatides of the liver by comparing the activity of the phosphatide P at the end of the experiment with the average activity of the inorganic P during the experiment, under conditions when the specific activity of the inorganic P of the liver does not change much. They reported the percentage of newly formed phosphatides in the liver of the rat in 4 hours to be 20% with a strong variation from animal to animal. However, the specific activity of the inorganic phosphorus did in fact change considerably during the course of our experiments. Moreover, even if the hepatic inorganic phosphorus, considered as precursor, remains constant, it is still necessary in accordance with the formulation of Zilversmit and associates to subtract the mean activity of the phospholipid P itself.

The method used for calculating R is based on the assumption that the hepatic inorganic phosphate is the immediate and only precursor of phospholipid phosphorus or, more to the point, that its specific activity is not materially different from that of the immediate precursor. Evidence that this condition is fulfilled with sufficient closeness to yield a reliable estimate of R is given if the value of R, when calculated for successive intervals of the experiment, is reasonably stable and shows no time trend. Still more convincing evidence is provided if the calculated value is essentially the same, even if the shape of the activity time curves themselves is markedly altered by changing the rate of administration of the activated material. As will be seen later, both criteria were used in the experiments here reported, and both confirmed the values obtained.

Experimental methods. Adult male white rats weighing approximately 200 g maintained on the stock commercial diet of "friskies" were fasted for 20 hours prior to the administration of P^{32}, and during the subsequent interval before removal of specimens. The P^{32}, obtained from the Massachusetts Institute of Technology, was administered as the dibasic sodium phosphate. In the first series of experiments reported later each rat received by a single intravenous injection 12 microcuries of P^{32} and not more than 0.02 mg of P^{31}. In the second series the P^{32} was administered by continuous intraperitoneal injection at the rate of 3 microcuries per hour. At specified intervals after the first administration the rats were anesthetized with pentobarbital sodium. Blood was withdrawn by cardiac puncture into a heparinized syringe. The liver was quickly removed and frozen

[2] Hevesy, G., and Hahn, L., *Kgl. Danske Videnskab. Selskab, Biol. Medd.*, 1940, **15**, 66.

[3] Artom, Camillo, Sarzana, Gaetano, and Segrè, Emilio, *Arch. Int. Physiol.*, 1938, **47**, 245.

[4] Chaikoff, I. L., *Physiol. Rev.*, 1942, **22**, 291.

[5] Hahn, L., and Tyren, H., *Arkiv f. Kemi, Mineral. och Geol.*, 1946, **21** A, No. 11.

[6] Hevesy, G., *Arkiv f. Kemi, Mineral. och Geol.*, 1947, **24** A, No. 26.

TABLE I.
Values Obtained After a Single Intravenous Injection of 12 Microcuries of P^{32} and Not More Than 0.02 mg P^{31}.*

1	Hr after administration, t	0.5	1	2	4
2	Rats	7	7	10	21
3	Body wt, g	189 ± 4.9	188 ± 4.7	214 ± 6.3	202 ± 3.8
4	Liver wt, % of body wt	3.2 ± 0.2	3.5 ± 0.4	3.8 ± 0.2	3.3 ± 0.1
5	Mg P per 100 g liver — Plasma inorganic P	6.9 ± 0.4	6.8 ± 0.4	7.5 ± 0.4	7.9 ± 0.2
6	Hepatic " "	31.8 ± 2.1	30.2 ± 1.6	31.1 ± 1.3	33.0 ± 1.1
7	" lipid "	137 ± 3.0	139 ± 4.4	133 ± 5.7	137 ± 1.8
8	Specific activity at time t — Plasma inorganic "	3,876 ± 203	2,015 ± 262	1,174 ± 87	744 ± 33
9	Hepatic " "	3,063 ± 280	2,578 ± 377	1,837 ± 154	1,102 ± 62
10	" lipid "	64 ± 7	133 ± 14	245 ± 24	339 ± 18
11	Relative activity, hepatic lipid P: inorganic P	0.02	0.05	0.13	0.31
12	Mean specific activity to time t — Hepatic inorganic P	2,618	2,641	2,410	1,932
13	Hepatic lipid "	30	67	126	211
14	Diff.	2,588	2,574	2,284	1,721
15	Change of specific activity, lipid P per hr	128	133	122	85
16	R	0.049	0.052	0.053	0.049

* All quantities are means for the number of rats given in row 2; the values after the ± are the standard errors of the means. The mean specific activity to time t was obtained as explained in the text.

in a mixture of carbon dioxide ice and alcohol and then was pulverized between chilled steel blocks.

An aliquot of the liver was extracted with trichloracetic acid, with the usual precautions, and the inorganic phosphate was precipitated as the magnesium ammonium salt. Traces of some organic phosphates are included in this precipitate but the amount and the radioactivity of these do not materially affect the value obtained for either the amount or the specific activity. The phosphorus content was determined by the method of Fiske and Subbarow[7] and the radioactivity was measured with a Geiger-Muller counter of the immersion type.[8] Specific activity is reported as counts per second per mg P after correction for decay from the time of injection to the time of counting. Direct measurements of the inorganic phosphate in a trichloracetic acid extract of the plasma were made without the preliminary precipitation.

Approximately 3 g of pulverized frozen liver were ground immediately with sufficient anhydrous Na_2SO_4 to remove the water.[9,10] The tissue was then extracted overnight at room temperature with 50 ml of alcohol-ether. The extract was filtered, the residue was washed once with alcohol-ether and twice with ether and the volume was made up to 100 ml. This procedure in the presence of plasma or tissue protein completely separates lipid P from inorganic P. Samples of blood or tissue taken a few minutes after injection of inorganic P^{32} show no radioactivity in the lipid fraction when the activity of the inorganic P is highest. The P^{32} was measured in a suitably diluted aliquot; the P^{31} was determined in duplicate aliquots after preliminary ashing with H_2SO_4 and H_2O_2. A large aliquot was evaporated to dryness by aeration at room temperature in the presence of a trace of hydroquinone for gravimetric determination of total lipid. Since at least 90% of the P in the dried lipids was found to be chloroform-soluble and since inorganic phosphate was not present, the alcohol-ether extracts were considered satisfactory for these studies. It must, however, be stated that, if the dried liver residue was extracted with boiling alcohol in the Bailey-Walker extraction apparatus for a 3 hour period, 20 mg more of chloroform-soluble P per 100 g of wet liver were obtained. This fraction, which probably includes the phospholipid attached to nucleoprotein described by Green-

7 Fiske, C. H. and Subbarow, Yellapragada, *J. Biol. Chem.*, 1925, **66**, 375.

8 Wang, J. C., Marvin, J. F., and Stenstrom, K. W., *Rev. Scient. Instruments*, 1942, **13**, 81.

9 Fairbairn, Donald, *J. Biol. Chem.*, 1945, **157**, 645.

10 Channon, H. J., Platt, A. P., and Smith, J. A. B., *Biochem. J.*, 1937, **31**, 1736.

TABLE II.
Values Obtained with Continuous Intraperitoneal Injection of P^{32} at Rate of 3 Microcuries per Hour.*

1	Hr after starting administration, t		1	2	3	4	6
2	Rats		1	3	2	3	2
3	Body wt, g		202	200	209	199	202
4	Liver wt, % of body wt		3.1	3.7	3.4	3.7	3.5
5	Mg P per 100	Plasma inorganic P	6.4	7.3	6.4	7.1	6.4
6	g liver	Hepatic " "	29.6	26.7	32.9	26.5	31.1
7		" lipid "	144	121	122	129	127
8	Specific activity	Plasma inorganic "	1,127	1,302	1,308	1,673	1,710
9	at time t	Hepatic " "	534	763	1,170	1,509	1,758
10		" lipid "	13	33	102	123	273
11	Relative activity, hepatic lipid: inorganic P		0.024	0.043	0.087	0.082	0.155
12	Mean specific	Hepatic inorganic P	262	470	657	824	1,093
13	activity to	" lipid "	6	15	31	53	105
14	time t	Diff.	256	455	626	771	988
15	Change of specific activity, lipid P per hour		13	17	34	31	46
16		R	0.051	0.037	0.054	0.040	0.046

* The explanation given in the footnote to Table I applies also to Table II.

stein[11] and which requires boiling alcohol for extraction, generally had a little less radioactivity than did the phospholipids extractable with alcohol-ether at room temperature.

Results. Two series of experiments were performed. In the first, which employed the larger number of animals, the radioactive material was injected intravenously in a single dose; in the second the material was administered by continuous intraperitoneal injection. The ratio of liver weight to body weight, the water content, total lipid extractable with alcohol-ether at room temperature, and the concentration of inorganic phosphate of the plasma were similar and within normal limits in all the animals. The chief figures of interest are summarized in Table I for the group of animals that received a single injection and in Table II for the group that received a continuous injection. The corresponding activity-time curves for hepatic inorganic phosphorus and hepatic phospholipid phosphorus were obtained by graphic smoothing of the observed specific activities in order to depict the course of the changes in these values and also for the calculation of the mean activities. The curves are shown in Fig. 1 and 2. The mean specific activity, for inorganic phosphorus and phospholipid phosphorus, which is required for the calculation of R, was obtained by evaluating the area under the time curve and relating this to the time interval considered. The area may be calculated by counting squares on the graph paper, by planimeter, or any other appropriate scheme. We have found most convenient and accurate the division of the area into strips and the use of Simpson's rule. The figures pertinent to the calculation of R are to be found in rows 12 through 16 of the tables. The rate of change of specific activity of hepatic phospholipid per hour (row 15) is obtained by dividing the specific activity at time t (row 10) by the time (row 1). The mean specific activities of hepatic inorganic P and hepatic phospholipid P (rows 12 and 13) were obtained from the area under the time activity curves, as explained previously, and their difference is given in row 14. The value of R (row 16), the proportional turnover rate for the period up to t is

$$R = \frac{\text{Change in specific activity of phospholipid P per hour}}{\text{Mean specific activity of inorganic P} - \text{mean specific activity of phospholipid P}}$$

These were calculated for the first experiment for ½, 1, 2 and 4 hours; the corresponding values were 0.049, 0.052, 0.053 and 0.049 respectively, the last being the rate calculated for the experiment as a whole. The rates calculated for the separate intervals 0 to ½,

[11] Greenstein, J. P., Nucleoproteins, in Anson, M. L., and Edsall, J. T., *Advances in Protein Chemistry*, New York, Academic Press, Inc., 1944, **1**, p. 245.

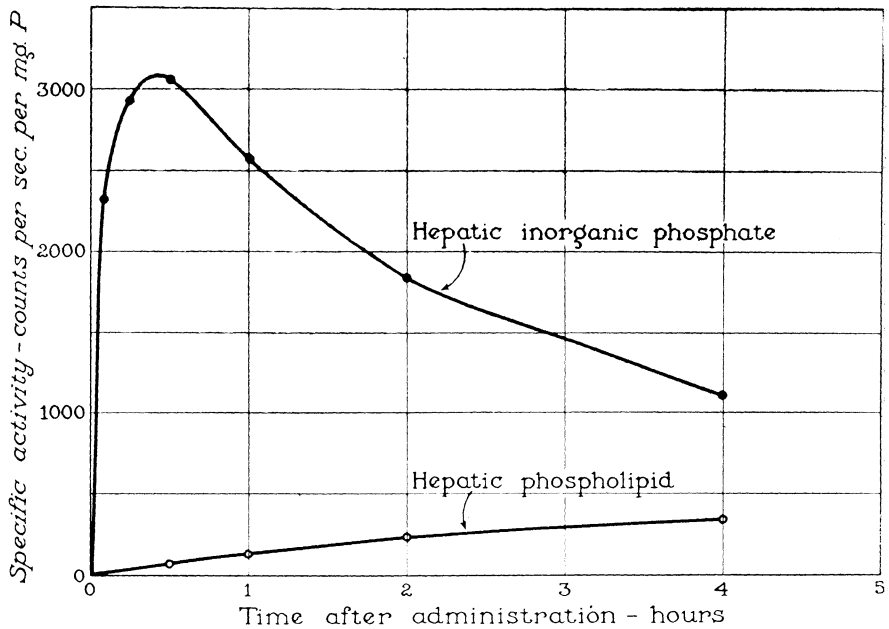

FIG. 1.

Curve of mean values of specific activities found after a single intravenous injection of phosphate P^{32}.

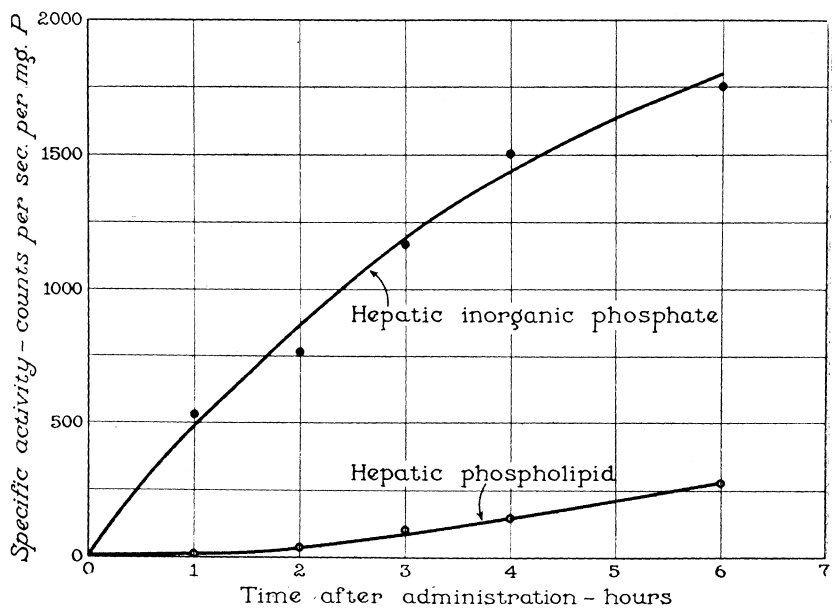

FIG. 2.

Curve of mean values of specific activities found with a continuous intraperitoneal injection of P^{32}.

½ to 1, 1 to 2, 2 to 4 hours were 0.049, 0.052, 0.053, 0.045. It is seen that this experiment yielded very consistent estimates for R in each of the periods. This is in contrast to the relative activity (ratio of specific activity of phospholipid P to that of inorganic P) which rose steadily in this period.

The results for the second series of experi-

ments, in which the injection of the radioactive material was continuous, are shown in Table II and the time-activity curves are shown in Fig. 2. It is to be noted that the curves are markedly different from those of the series with the single injection. This result is to be expected. The calculated values of R are given as for the periods up to 1, 2, 3, 4 and 6 hours; these were respectively 0.051, 0.037, 0.054, 0.040 and 0.046, the last representing the result for the experiment as a whole. The figures are not quite so consistent as those for the first series but the variation is not larger than is to be expected, considering the smaller number of animals used in this series. The relative activity shows no consistency with the first experiment. The figure for R provided by the entire experiment, 0.046, agrees very well with that obtained in the first experiment, 0.049.

Conclusions. The proportional turnover rate R for hepatic phospholipid P in the white rat is on the average close to 5% per hour, and since the mean concentration of hepatic phospholipid P for both experiments together was 132 mg per 100 g of liver, that r, the mass turnover rate, is about 6 mg P per hour per 100 g of liver, which is 0.2 mg P per 100 g body weight.

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Growth of Normal Male and Female Mice and of Female Mice Bearing Ovarian Grafts.*†

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It has been clearly shown that mouse ovaries, under certain conditions, can supply androgens of sufficient quantity and quality to maintain, in normal conditions, the accessory sex glands of castrate male mice.[1,2] It has been proven that environmental temperature is a factor in causing grafted ovaries to elaborate androgens, but the exact androgens secreted have never been determined.

It was suggested to the author by Doctor Carl G. Hartman that perhaps female mice bearing active androgenic ovarian grafts might assume a growth pattern comparable to that of normal males. Following this suggestion a group of our animals in experiment were weighed twice weekly, and the weights obtained are presented in Fig. 1. Five normal males and 10 normal females were used as control animals. Twenty-eight females bearing autografts of ovaries in their ears comprised the experimental group. All of the animals were of the same age when started on the experiment. It will be noted that the normal males averaged 16.6 g at the outset of the experiment, while the normal and experimental females averaged 14.9 g. The weights were recorded and plotted for approximately 250 days. At the end of this period the plateau of growth was essentially complete.

A difference of slightly less than 2 g at the outset of the experiment, between males and females, had increased to approximately 4.5 g 60 days later. The difference of 4-4.5 g in the weights of the 2 groups was maintained from then till the termination of the experiment. At no time did the curve obtained

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† The mice used in these experiments were the CHI inbred strain obtained from Dr. L. C. Strong.

1 Hill, R. T., *Endocrinology*, 1937, **21**, 633.

2 Hill, R. T., and Strong, M. T., *Endocrinology*, 1938, **22**, 663.