found that during daily injection of estrone in pregnant rabbits beginning on the 29th day 400 I.U. killed the young but did not significantly delay parturition and 1000 I.U. prevented delivery; delivery occurred normally on the 32nd day in this strain of rabbits. The prevention of parturition in the rabbit was regarded as due primarily to prolongation of the functional state of corpora lutea. The prolongation of pregnancy in the hypophysectomized rabbit[10] and in the rat[11] by treatment with estrogens was similarly interpreted. Our findings that estrone injection started on the 19th day of pregnancy in the rat brought about vigorous but ineffective efforts to deliver young while progesterone treatment started on the same day induced quiescence, suggest that estrone plays a role in the prevention of parturition in the rat other than by way of its action on the corpora lutea. Because estrone apparently increases the frequency and intensity of contraction of abdominal muscle, it is suggested that failure to deliver the young is due to obstruction of the birth canal possibly as a result of failure of relaxation in the region of the cervix. In the rat wide abdominal incision during parturition practically stops transport and expulsion of the fetuses and closure of the incision restores both processes.[3] This observation, which was interpreted as evidence that contractions of the diaphragm and abdominal muscles provide the primary force for delivery of young, supports our view that failure of expulsion in estrone-treated rats showing vigorous labor is due to failure of uterine relaxation.

Our observation that progesterone injection started simultaneously with estrone treatment on the 19th or 20th day of pregnancy prevents labor, uterine bleeding and death of young suggests that the death of fetuses induced by estrone may be due, at least in part, to intense but ineffective labor.

Summary. Progesterone (2.5 mg daily) beginning on the 19th day of pregnancy in intact rats prevents labor, uterine bleeding and parturition. Estrone (25 γ) started on the 19th or 20th day induces uterine bleeding, vigorous but ineffective labor and death of fetuses. Estrone (100 γ) begun on the 20th day in 1 animal brought about delivery of young 1 day premature. Spontaneous delivery occurred in 4 rats given 25 γ of estrone about 24 hours before time for delivery. Progesterone (2.5 mg) given simultaneously with estrone (25 γ) beginning on the 20th day has the same effect as progesterone alone, preventing the above effects of estrone alone. It is suggested that estrone plays a role in the prevention of parturition in the rat other than by way of its action on the corpora lutea.

Progesterone from Schering Corporation, Bloomfield, N. J., and estrone from Parke Davis and Co., Detroit, Mich., were generously supplied for this study.

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17179. Urinary Excretion of Phosphate Following the Injection of Sodium p-Aminohippurate.

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In the course of recent studies of osmotic diuresis in man,[1] the rate of urinary excretion of phosphate was found to be increased following the injection of sodium p-aminohippurate. Although PAH has been found to

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[1] Rapoport, S., West, C. D., and Brodsky, W. A., *Am. J. Physiol.*, in press.

TABLE I.
Effect of NaPAH on Urinary Phosphate Excretion.

Period No. and specimen		Concurrent time, min.	Urine volume, cc/min./ 1.73 M^2	PAH conc., mg %	Phosphate conc., m.Osm/L	Phosphate excreted, m.Osm/min./ 1.73 M^2
L.J. Hydration. 9 yrs. S.A. 1.25 M^2. Wt. 37.3 kg.						
P–1	U	−66 to −35	1.10		12.3	.014
P–2	U	−35 to −10	8.39		2.0	.017
	P	−1			1.50	
		0 to 10 I.V. injection 50 cc 20% NaPAH.				
1	U	24 to 58	3.31	3800	11.0	.036
	P	28		62.2	1.54	
2	U	58 to 75	4.61	1529	6.8	.031
	P	69		14.1	1.56	
3	U	75 to 97	6.13	739	4.4	.027
4	U	97 to 118	8.00	254	3.0	.024
W.H. Dehydration. 11 yrs. S.A. 1.12 M^2. Wt. 30.9 kg.						
P–1	U	−116 to −86	0.38		53.2	.020
P–2	U	−86 to −59	0.31		59.4	.018
P–3	U	−59 to −23	0.28		67.8	.019
	P	−1			1.61	
		0 to 5 I.V. injection 35 cc 20% NaPAH.				
1	U	22 to 42	2.88	4560	19.4	.056
	P	25		54.3	1.54	
2	U	42 to 63	1.63	4622	28.7	.047
	P	56		13.6	1.52	
3	U	63 to 82	0.82	4114	46.4	.038

affect the renal excretion of several substances,[2-4] an effect on phosphate excretion has not been previously described. This report concerns studies of phosphate excretion at various plasma PAH levels under conditions of both hydration and dehydration.

Procedure and methods. The studies were done on one female and 5 male patients, 9 to 12 years of age, who showed no evidence of renal disease. One child had slight albuminuria and hematuria associated with an episode of acute rheumatic fever three weeks previous to the experiment but subsequently has shown no evidence of renal disease. The subjects were in the post-absorptive state. Two subjects were deprived of water for 16 hours prior to the procedure. In these the PAH produced osmotic diuresis. In the other patients, high urine flows were maintained by water intake as is commonly done for renal clearance studies.

The general procedure of 4 experiments was as follows: After the collection of several preliminary urine specimens and one blood specimen, sufficient 20% NaPAH was injected intravenously in a 10 to 15 minute interval to produce plasma levels of 60 mg % or higher. Four to six urine specimens at 15 to 20 minute intervals and 2 or more blood specimens were then collected.

In 2 of the hydrated subjects, the procedure was somewhat modified as will be described later.

In blood and urine, PAH was determined by the method of Smith *et al.*[5] and phosphate by the method of Fiske and Subbarow.[6] Urine volumes were corrected to 1.73 M^2 for the calculation of minute phosphate excretion.

Results. The pertinent data of 2 representative experiments during hydration and dehydration are given in Table I. For all experiments, phosphate excretion during preliminary urine periods prior to the injection of NaPAH ranged from 0.002 to 0.020 mOsmols/min. In every case the rate of ex-

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[3] Beyer, K. H., Woodward, R., Peters, L., Verwey, W. F., and Mattis, P. A., *Science*, 1944, **100**, 107.

[4] Houck, C. R., PROC. SOC. EXP. BIOL. AND MED., 1946, **63**, 398.

[5] Smith, H. W., Finkelstein, N., Aliminosa, L., Crawford, B., and Graber, M., *J. Clin. Invest.*, 1945, **24**, 388.

[6] Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.*, 1925, **66**, 375.

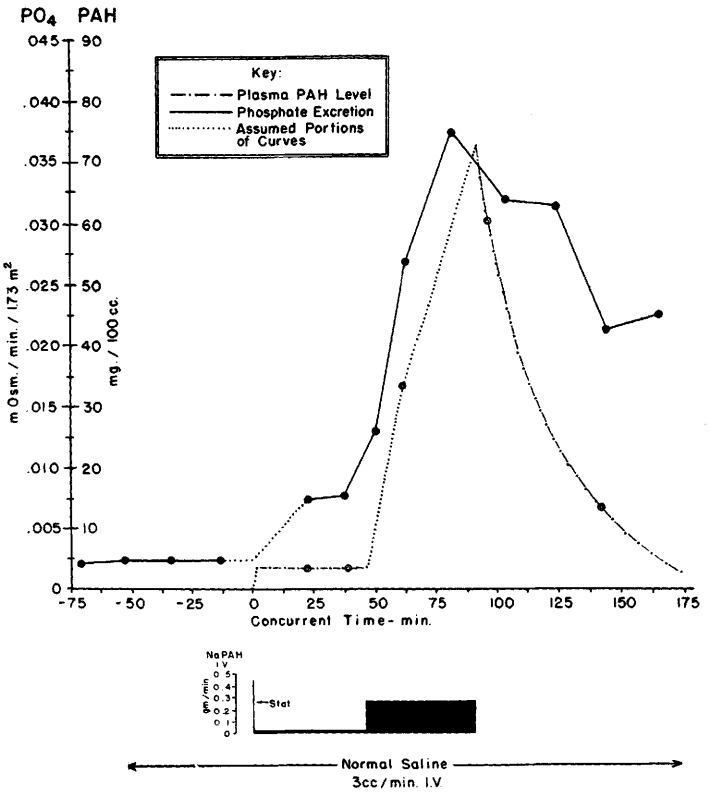

FIG. 1.
Phosphate excretion and plasma PAH level. See text for details.

cretion increased following the injection of NaPAH, regardless of the state of hydration of the subject. The maximum rate usually occurred during the first or second collection period following the rapid injection, not always coincident with the highest plasma PAH concentration. The maximum rate of phosphaturia ranged from 0.017 to 0.069 mOsmols/min., representing 2.3 to 16 fold increases over the respective rates during preliminary periods. After reaching a maximum, the excretion diminished at a rate coinciding roughly with the fall in plasma PAH concentration.

Plasma phosphate concentration did not change significantly during the course of the experiments. The phosphate level averaged 1.40 mOsmols/liter for six specimens taken prior to the injection of NaPAH and 1.46 mOsmols/liter for 19 specimens taken after the injection.

In Fig. 1 is presented one of two experiments so arranged as to observe phosphate excretion at constant low plasma PAH levels (3 to 5 mg %) and during the rise to and fall from higher concentrations. Throughout the experiment, with the exception of the first two preliminary periods, normal saline was injected intravenously at a constant rate of 3.0 cc/min. with sufficient NaPAH added at intervals to produce the plasma concentrations shown. The effect of PAH in increasing the rate of phosphate excretion is readily apparent.

Discussion. Under normal conditions extensive tubular reabsorption of phosphate takes place. Assuming a filtration rate of 120 cc/min., the amount of phosphate excreted during the preliminary periods of the experiments here presented averaged 7% of the amount filtered. After injection of NaPAH during the periods of greatest phosphaturia, assuming the same filtration rate, the phosphate excreted ranged from 9 to 45%, with an average of 26% of the amount filtered. The inhibition of tubular phosphate reabsorp-

tion by PAH is thus of considerable magnitude but is not sufficient to cause excretion to occur at the level of glomerular filtration.

It is worthy of note that the diminished tubular reabsorption of phosphate which occurs after injection of parathyroid hormone is of about the same order of magnitude. The data of Harrison and Harrison[7] on dogs show that after injection of parathyroid extract, 25 to 28% of the filtered phosphate was excreted. Filtration rates were determined by creatinine clearance in these experiments. In normal adults the ratio of excreted to filtered phosphate after parathyroid hormone injection can be calculated from the data of other workers, assuming a filtration rate of 120 cc/min. The 5 subjects of Ellsworth and Howard[8] excreted 11 to 41% of the phosphate filtered in the second and third hours after injection of 40 units of parathyroid hormone. Essentially the same percentage of phosphate excreted may be calculated from the data for the two subjects of Goadby and Stacey[9] after doses of 60 and 100 units of parathyroid extract.

Previous work has shown that the tubular transport of a number of substances may be depressed by PAH. The tubular reabsorption of ascorbic acid[2] and the tubular excretion of penicillin[3] are reduced by the presence of high plasma PAH levels. The inhibition of tubular transport of these substances may be so great that excretion occurs at the level of glomerular filtration. Glucose and PAH appear to inhibit reciprocally the tubular transfer of each other. In dogs the T_m for glucose has been shown to be consistently depressed by high PAH levels.[4] In man, the opposite effect, an increased T_m for glucose in the presence of high PAH levels, has been reported by Klopp, Young, and Taylor[10] in 2 out of 4 patients studied. An explanation of this unexpected finding may lie in the fact that in their experiments, the mannitol clearance, used as a measure of glomerular filtration rate, was found to be increased significantly with high PAH levels. It is possible that the augmentation of the mannitol clearance and consequently of the glucose T_m was the result of an error in the determination of mannitol due to interference by PAH. Barker and Clark[11] have recently shown that in the method used by Klopp *et al.* (periodate-iodide-thiosulfate titrimetric procedure), PAH causes a large positive error, to the extent of 0.27 mg of mannitol per mg of PAH. The reciprocal effect, *i.e.*, inhibition of the transport of PAH by high plasma glucose levels has been consistently demonstrated in both dog and man.[4,10,12,13]

The nature of the inhibition of tubular transport can only be speculated upon. The inhibition of creatine reabsorption by glycine, alanine and glutamic acid[14,15] and of xylose reabsorption by glucose[16] has been attributed to competition for a common mechanism of limited capacity involving a single transport substance (B substance of Shannon[17]). Although such an explanation may be valid for structurally similar substances, it is difficult to conceive of one enzymatic or chemical mechanism sufficiently versatile to act in such a widely differing manner as to secrete PAH and penicillin or reabsorb ascorbic acid, glucose, and the phosphate ion. The circumstance that 4 of the 5 substances just mentioned are anions would suggest the existence of a tubular transport mechanism based on this property. However, the mutual inhibition of glucose and PAH speaks strongly against such an assumption. Another point, possibly contradicting the idea of a common mechanism, is the fact that one of the sub-

[7] Harrison, H. E., and Harrison, H. C., *J. Clin. Invest.*, 1941, **20**, 47.

[8] Ellsworth, R., and Howard, J., *Bull. Johns Hopkins Hosp.*, 1934, **55**, 296.

[9] Goadby, H. K., and Stacey, R. S., *Biochem. J.*, 1934, **28**, 2092.

[10] Klopp, C., Young, N. F., and Taylor, H. C., Jr., *J. Clin. Invest.*, 1945, **24**, 117.

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[12] Grimelli, L. J., Chertack, M. M., Rhetta, H. L., Kendrick, A. B., and Forrest, R. A., *J. Lab. and Clin. Med.*, 1948, **33**, 1617.

[13] Kelley, V. C., and McDonald, R. K., *Am. J. Physiol.*, 1948, **154**, 201.

[14] Pitts, R. F., *Am. J. Physiol.*, 1943, **140**, 156.

[15] Pitts, R. F., *Am. J. Physiol.*, 1944, **140**, 535.

[16] Shannon, J. A., *Am. J. Physiol.*, 1938, **122**, 775.

[17] Shannon, J. A., *Physiol. Revs.*, 1939, **19**, 63.

stances, phosphate, is an inorganic ion.

The inhibition of tubular transport by PAH may be more adequately explained by the hypothesis of a common energy source of limited capacity. Competition for the available energy by the activity of the mechanism for the transport of one solute would then result in diminished transport of another solute. Neither the locus nor the character of the energy limitation can be stated. Some of the possible mechanisms involved have been discussed by Selkurt[2] and by Houck.[4]

It should be emphasized that the inhibition of tubular transport of one substance by another may result in serious error when several renal functions are measured simultaneously.

Summary. The urinary excretion of phosphate is increased by the injection of sodium p-aminohippurate in man. The extent of the phosphaturia tends to vary directly with the plasma PAH level. At high PAH levels, the amount of phosphate excreted averages 26% of that filtered, a proportion comparable in magnitude to that reported after injection of parathyroid hormone.

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17180. Effect of Dietary Cholesterol on the Metabolic Rate of Hyperthyroid Rats.*

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It was recently observed that dietary cholesterol increases the survival time of rats fed toxic doses of thyroid hormone.[1,2] In an attempt to learn whether this protective action of cholesterol is correlated with a corresponding modification of the effects of thyroid on the metabolic rate, the influence of dietary cholesterol was investigated on the oxygen consumption of hyperthyroid rats.

Methods. Thirty-two male rats (USC strain), about 9 weeks of age, were distributed evenly among 4 groups which received the following diets *ad libitum*: I. This laboratory's stock diet, slightly modified to contain more vitamin B complex‡ ("N"). II. Diet I, containing, in addition, 1 to 2% cholesterol§ ("C"). III. Diet I, containing, in addition, 0.15% desiccated thyroid U.S.P.§ ("T"). IV. Diet I, containing, in addition, both 0.15% thyroid and 1 to 2% cholesterol. ("T + C"). At first, cholesterol was fed at a level of 1%, but after 3 weeks, its concentration in the diet was raised to 2%. The cholesterol was dissolved in hot cottonseed oil, replacing a corresponding amount of oil in the diet. In order to increase cholesterol absorption, 0.25% bile salt§ was added to all diets.[3]

The oxygen consumption was determined using a closed circuit apparatus provided with separate chambers for individual animals.[4] After repeated measurements during a preliminary training period of 5 weeks, feeding

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1 Marx, W., Meserve, E. R., and Deuel, H. J., Jr., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 385.

2 Ershoff, B. H., and Marx, W., *Exp. Med. and Surg.*, 1948, **6**, 145.

‡ Whole wheat, 32.5%; oats, 32.75%; skimmed milk, 10%; alfalfa meal, 4%; yeast, Anheuser-Busch strain G, 9.5%; Wesson oil, 8%; fortified oil containing 1500 I.U. vitamin A/g and 160 I.U. vitamin D_2/g, 2%; sodium chloride, 0.5%; calcium carbonate, 0.5%; bile salt, 0.25%.

§ Cholesterin c.p., Amend Drug and Chemical Co., New York City; desiccated thyroid, U.S.P., Armour and Co., Chicago, Ill.; sodium glycocholate (pure), City Chemical Corp., New York City.

3 Schoenheimer, R., *Biochem. Z.*, 1924, **147**, 258.