volitional effort but without the complicating responses of other units.

A complete discussion of functional and technical aspects of the experiments will not be undertaken here but Fig. 1 indicates the type of record which has been obtained. The record has been traced in ink to facilitate reproduction. Two steel sewing needles (No. 12), lacquered to the extreme tips, led to the grids of a differential amplifier. An unlacquered needle was grounded. The 3 needles were inserted close together in the brachialis muscle. The heel of the hand rested on the diaphragm of a tambour and served to record a slight flexor movement of the forearm. During the first part of the record, discrete volitional efforts at intervals of about 0.8 sec each resulted in a single discharge of the single motor neurone which innervated the group of muscle fibers close to a recording electrode. Other motor neurones may or may not have sent impulses to other muscle fibers less favorably situated for recording. Somewhat quicker movements caused the appearance of double spikes due either to repetitive discharge of the original unit or to the introduction of potentials from a second nearby motor unit which had previously been inactive. With sustained effort the usual rhythmic discharge is recorded.

It has been generally believed that the simplest volitional movement possible involves a brief but rhythmic discharge of impulses from the anterior horn cells concerned. The present studies indicate that when the volitional movement is sufficiently slight and brief, the motor neurones may discharge only once.

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Retention of Orally Administered Radio-Phosphorus by Mice.*

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This experiment was devised to determine if the whole body retention of orally administered radio-phosphorus could be increased or decreased by the simultaneous administration of various foods or

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salts. By increasing the amount of radio-phosphorus absorbed, less would be required orally to build up a given concentration in tissues of patients suffering with various generalized neoplastic and near-neoplastic diseases and to whom radioactive agents are given in therapeutic attempts.

Methods and Materials. Three groups of closely related Swiss mice which totaled 190 in number and which were of 30, 70 and 250 days of age respectively, and an additional group of 64 highly inbred mice of the C.B.A. strain, which were 120 days old, were used. All of the 254 mice were placed in cages with wire floors to prevent coprophagy. The males and females were equally divided in each experiment and separated.

Phosphorus. The radioactive phosphorus was produced for us by the Lawrence and Cooksey[1] cyclotron and was converted into neutral isotonic sodium phosphate. 0.25 cc containing 3.7 mg of sodium phosphate and emitting beta radiation equivalent to 5 microcuries,† when administered, was given by stomach tube to each of the Swiss mice, and 0.5 cc containing 7.5 mg Na_2HPO_4 and emitting 2.86 microcuries was given to each of the C.B.A. mice. A small and undetermined amount of naturally occurring non-radioactive phosphorus was present in the food supplements, which were simultaneously administered.

Diet. The regular diet consisted of Purina dog chow *ad lib.*, oats and lettuce. This was supplied to all the mice previous to the fasting periods of 20-24 hours and immediately after the administration of radio-phosphorus and the accompanying foods and salts. The carbohydrates—glucose which requires phosphorylation for absorption and mannose that does not—were given in solutions of 10% concentration which are optimum for maximum speed of absorption.[2] Glycine, which is a simple amino acid, was administered in a solution of 10% concentration and raw egg albumen of 50% concentration. The fats—saturated lauric acid, unsaturated oleic acid and a good quality of fortified cod liver oil containing vitamins A, B, D and G—were administered in 0.5 cc doses. See table for concentrations. Ferric and ferrous sulfate were given in doses comparable with those prescribed for human beings. From 6 fasting female mice of one group, 1 cc of blood was withdrawn from the tails just prior to the administration of radio-phosphorus and glucose.

All of the animals were sacrificed at the end of a 72-hour period,

1 Lawrence, E. O., and Cooksey, D., *Phys. Rev.*, 1936, **50**, 1131.

† Uranium standard.

2 Verzar, F., *Absorption from the Intestines*, Longmans, Green and Company, 1936.

TABLE I.

	No. of mice	Age (days)	Avg g wt	Amt and type of food or agent administered simultaneously with the radio-phosphorus per mouse	Amt of P^{32} (μ/mouse) retained at end of 3-day period‡	Avg % of retention per mouse	% of retention per g of mouse
Swiss*							
Young	20	30	16.1	.25 cc H_2O	1.30	26	1.6
	19	30	16.8	*Ad lib.*, regular diet	1.44	28.8	1.71
	17	30	16.7	.25 cc 10% sol. of glucose	1.25	25.0	1.5
	19	30	15.6	.25 cc aq. sol. ferrous sulfate (10 mg)	.71	14.2	.91
Adult	20	70	23.4	As above (water)	1.03	20.6	.88
	20	70	22.6	" " (regular diet)	1.35	27.0	1.19
	16	70	20.7	" " (glucose)	1.37	27.4	1.32
	18	70	21.6	" " (ferrous sulfate)	.61	12.2	.57
Old	10	250	29.0	" " (water)	1.45	29.0	1.00
adult	10	250	31.2	" " (regular diet)	1.59	31.8	1.02
	9	250	31.9	" " (glucose)	2.04	40.7	1.28
	6	250	31.9	" " (ferrous sulfate)	.54	10.8	.34
	1 cc of blood was withdrawn from the tails of the 6 females below.						
	6	70	19.2	.5 cc of 10 % sol. of glucose	1.53	30.6	1.58
Total	190						
C.B.A.†							
Adult	10	120	19.7	.5 cc water	.97	34.0	1.72
	10	120	21.5	.5 " of 10% sol. glucose	1.07	37.4	1.72
	10	120	20.0	.5 " of 10% sol. glucose	1.24	43.5	2.18
	5	120	22.6	.5 " of 10% sol. mannose	.97	34.0	1.50
	5	120	22.2	.5 " of 50% sol. egg white	.96	33.6	1.51
	5	120	20.6	.5 " cod liver oil	1.07	37.4	1.81
	5	120	21.6	.5 cc aq. susp. lauric acid (35 mg)	.87	30.4	1.40
	5	120	20.5	.5 cc aq. susp. oleic acid (100 mg)	1.13	39.6	1.93
	5	120	16.5	.5 cc aq. sol. ferrous sulfate (10 mg)	.51	17.8	1.08
	4	120	18.2	.5 cc aq. sol. ferric sulfate (10 mg)	.73	25.5	1.41
Total	64						
Grand Total	254						

SUMMARY.

Weighted avg % per g retention of P^{32} disregarding age.

No. of mice	Agent used	%
60	Water	1.26
49	Regular diet	1.30
52	Glucose	1.71
48	Ferrous sulfate	.82
Total 209		

*Each of the Swiss mice received 5 microcuries of radiophosphorus in .25 cc of isotonic Na_2HPO_4 by stomach tube.

†Each of the C.B.A. mice received 2.86 microcuries of radiophosphorus in .5 cc of isotonic Na_2HPO_4 by stomach tube. All mice were fasted 24 hours before administration of radiophosphorus except those Swiss mice to whom regular diet was given.

‡Corrected for decay of radiophosphorus.

since it was assumed that all of the unabsorbed radio-phosphorus would have been excreted from the gastro-intestinal tract during that interval of time. The bodies of the mice in each experiment were pooled and placed in large crucibles and ashed at 520°C. The ash was then thoroughly mixed and an aliquot portion assayed for radioactivity. The results of the assay were corrected for the rate of decay of the radioactive phosphorus, the half-life of which is 14.3 days, to the date of administration.

Results. (See Table I.) The results are discussed in relation to the strain of mice used, since the rate of metabolism (presumably) differs in the 2 strains.

a. The 190 Swiss mice were divided into 3 groups—young, adult and old adult. The control animals, those that received labelled phosphorus alone, retained respectively 1.6%, 0.88% and 1.00% per gram of body weight of the radio-phosphorus administered, at the end of the third day; those maintained on a regular diet 1.71%, 1.19% and 1.02% respectively; those given glucose simultaneously with the radio-phosphorus 1.5%, 1.32% and 1.28% respectively, while those to whom ferrous sulfate was administered retained only 0.91%, 0.57% and 0.34% respectively. It is apparent that the administration of glucose with the labelled phosphorus tends to increase the absorption[3] while ferrous sulfate markedly decreases it. The results indicate that the young mice tend to retain more of the radio-phosphorus per gram of whole body than the older groups.

b. The 64 C.B.A. mice were divided into 10 groups of varying numbers. Only in those groups of mice receiving glucose and unsaturated oleic acid, as supplements, were there significant increases in retention above the control group. The iron salts again were associated with decreased retention.

The weighted mean value for the retention at the end of 3 days by all 60 of the control mice who received no supplement was 1.26% of the phosphorus administered, per gram of whole body. The mean retention by the 52 mice who received glucose supplement was 1.71% per gram while the 49 mice on regular diet retained 1.30% per gram. For the 48 mice receiving ferrous sulfate, the mean retention was 0.82% per gram.

In the small group of 6 female mice there was an indication that active hematopoiesis tended to increase the retention of radio-phosphorus.

The following experiment was devised to determine the differences

[3] Laszt, L., Intestinal Sugar Absorption and Phosphate Metabolism. *Nature*, 1940, **145**, 899.

TABLE II.

	No. of mice	received	Avg retention of radiophosphorus in % of dose per g of whole mouse on seventh day after administration
(Each mouse received .006 mg Na_2HPO_4) A	2	NaCl and P^{32} intraperitoneally	2.85
	2	NaCl and P^{32} by stomach tube	2.1
(Each mouse received 7.5 mg Na_2HPO_4) B	2	P^{31} and P^{32} intraperitoneally	1.0
	2	P^{31} and P^{32} by stomach tube	1.2

in retention when markedly different quantities of inert phosphorus accompanied the administration of radio-phosphorus.

0.2 cc of a sodium phosphate solution, each cubic centimeter containing 0.3 mg sodium phosphate and emitting beta radiation equivalent to 194 microcuries, was placed in 10 cc of distilled water. This solution was divided equally and to one half .045 g of sodium chloride was added and to the other .075 g of sodium phosphate. 0.5 cc of these isotonic solutions were given either by stomach tube or intraperitoneally to 8 closely related C.B.A. mice of same age and weight. Each mouse received 1.91 microcuries of radiation on the date of administration, but 4 (A) received 1250 times more phosphorus than the remaining 4 (B). Seven days later the bodies of the animals were assayed and the results corrected for decay of radioactive phosphorus. (Table II.)

Thus it is observed that the retention of radiophosphorus varies to only a limited extent when different amounts of inert phosphorus accompany it on administration.

In explanation of the results, it would seem that absorption of phosphorus takes place most readily in the upper regions of intestines because the glucose which is readily absorbed as a phosphorylated compound is associated with an increased retention when compared with the retention by mice fed food of the regular diet, which requires longer periods of time for assimilation and absorption. Radio-phosphorus may be absorbed by at least 2 possible mechanisms: (1) diffusion as inorganic phosphate, or (2) by phosphorylation with glucose, etc., present in the gastro-intestinal tract. Therefore, when glucose is administered simultaneously with phosphorus, the first and second mechanisms are operative, while, when phosphorus is administered alone, only the first is operative. There is some slight evidence in the results obtained from the C.B.A.

groups of mice that cod liver oil and oleic acid play rôles similar to glucose.

In the oral administration of radio-phosphorus, in therapeutic attempts, it would seem desirable to administer the P^{32} on an empty stomach and with a 10% solution of glucose in order to obtain greater absorption and retention. The simultaneous administration of iron salts should be avoided.

The greater retention of P^{32} in the young animals can be explained partially on the basis of a more rapid growth, particularly of the osseous system.

Summary. Glucose increased and iron salts decreased the absorption of radioactive phosphorus from the gastro-intestinal tract.

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Choline, Creatine and the "Labile Methyl" Supply.

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Hemorrhagic degeneration, characterized by renal hemorrhage and fatty livers, occurs in young rats on diets low in choline and in methylated compounds possessing choline-like activity.[1] Supplements of choline,[1] methionine[1] or betaine[2] readily prevent this dietary deficiency. The effect of creatine in decreasing the occurrence of renal lesions is shown in Table I. The liver fat is not significantly affected unless both creatine and choline are fed. Prevention of the fatty liver requires more choline than is necessary for the prevention of renal hemorrhage.[1] The experiments in Table I demonstrate that supplements of creatine decrease the severity of but do not prevent hemorrhagic degeneration. This effect differs from that of methionine and betaine in that only partial protection occurs and the degree of protection is not increased by larger supplements of creatine.

The following explanation, which deals with the transfer of essential methyl groups and not with the synthesis of the rest of a

1 Griffith, W. H., and Wade, N. J., *J. Biol. Chem.*, 1939, **131**, 567; 1940, **132**, 627.

2 Griffith, W. H., and Mulford, D. J., unpublished experiments reported to the Division of Biological Chemistry of the American Chemical Society in Detroit, Sept. 9 to 13, 1940.