TABLE I.
Comparative Toxicity of Magnesium Phenobarbital and Magnesium Phenobarbital Plus Phenacetin.

Dose Mg. Phenobarbital mg./K		No. Rats	Deaths 12 hr.	24 hr.	48 hr.	72 hr.	Total	%
200		30	1	—	1	—	2	7
225		30	—	5	—	—	5	17
250		10	3	—	1	—	4	40
275		10	3	2	—	—	5	50
300		10	3	1	—	1	5	50
Total		90	10	8	2	1	21	23.5
	Phenacetin mg./K							
200	1000	30	—	—	1	—	1	3
225	1125	30	3	—	1	1	5	17
250	1250	10	1	—	1	—	2	20
275	1375	10	2	—	—	—	2	20
300	1500	10	1	1	—	—	2	20
Total		90	7	1	3	1	12	13.5

with respect to the time of onset of hypnosis, the duration of anesthesia, and the recovery period.

Conclusion. The above results confirm for phenobarbital those reported by Loewe for barbital. It is apparent that when an analgesic is given simultaneously with a hypnotic drug, care must be exercised in the choice of the former. Thus, whereas acetyl salicylic acid antagonizes the hypnotic action of phenobarbital, phenacetin in no way diminishes its activity. Furthermore, the evidence shows that phenacetin may exert a protective influence by antagonizing the toxic effects of phenobarbital.

8209 C

Effect of Diet upon Blood Phosphorus Partition of Rats with and without Insulin.

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Beginning with the work of Harden and Young and followed by that of Embden and his associates, it has been evident that phosphorus may play some rôle in the oxidation of carbohydrate. This idea became more fixed after the isolation of insulin when it was shown that not only blood sugar but also urine phosphate and blood inorganic phosphorus decreased after the administration of this hormone. The drop in blood inorganic phosphorus following insulin injection has been observed by Wigglesworth, Woodrow, Smith and Winters[1] for normal rabbits, Briggs, Koechig, Doisy, and Weber[2] for normal dogs, and Harrop and Benedict[3] for normal and diabetic human beings.

Studying the different fractions of blood phosphorus during insulin action, Kay and Robison[4] found that, in the rabbit, there is little change in the lipoid phosphorus, an increase in total acid soluble, a slight decrease in the inorganic phosphorus, and therefore an increase in the organic portion. Kerr[5] reported, in a similar study on the partition of blood phosphorus in dogs under the influence of insulin, a drop in the inorganic phosphorus and variable changes in the lipoid fraction, although he was unable to find, as had Kay and Robison, an increase in the organic fraction.

The decrease of blood inorganic phosphorus after insulin injection has been taken by some to indicate a shift of phosphorus from blood to tissues where it may be held in combination with the sugar which simultaneously disappears from the blood. This suggestion, however, has not been borne out by the work of Kay and Robison,[4] and Cori and Goltz[6] who were unable to find an increase, respectively, in muscle or in liver organic phosphates after insulin. Total blood phosphorus determinations, which might help to throw some light on this problem, have not been reported.

Since there appear, then, to be no clear-cut results indicating the fate of the phosphorus which seems to disappear after insulin administration, and since so little work has been done on the changes in all of the phosphorus fractions under the influence of insulin, further study of these problems is indicated.

This experiment was undertaken in order to observe changes in the sugar, inorganic phosphorus, total acid soluble phosphorus, lipoid phosphorus, and total phosphorus of the whole blood of rats on 3 different diets when insulin was administered to one-half of the animals 30 minutes before blood samples were taken. In none of the previous studies has any attention been paid to the habitual

[1] Wigglesworth, V. B., Woodrow, C. F., Smith, W., and Winters, L. B., *J. Physiol.*, 1922-23, **57**, 447.

[2] Briggs, A. P., Koechig, I., Doisy, E. A., and Weber, C. J., *J. Biol. Chem.*, 1923, **58**, 721.

[3] Harrop, G. A., and Benedict, E. M., *J. Biol. Chem.*, 1924, **59**, 683.

[4] Kay, H. D., and Robison, R., *Biochem. J.*, 1924, **18**, 1142.

[5] Kerr, S. E., *J. Biol. Chem.*, 1928, **78**, 35.

[6] Cori, C. F., and Goltz, H. L., *Am. J. Physiol.*, 1925, **72**, 256.

diet of the subjects, although it is well known that both blood sugar and blood phosphorus levels of animals in the post-absorptive condition may be affected by the composition of the diet.

The 3 diets, designated respectively as standard, high carbohydrate, and high fat, were planned to contain, as nearly as possible, comparable amounts of agar, cottonseed oil, protein, and salt mixture per calorie, as shown in Table I. In each case the diets were supplemented with 5.5 gm. of dried brewers yeast and 12 drops of codliver oil per animal per week.

TABLE I.
Composition of Basal Diets.

Diet	Constituents % by weight		% of Calories	Nitrogen %	Phosphorus %
Standard				2.6	0.38
	Casein	20.0	17.9		
	Cornstarch	57.9	51.9		
	Hydrogenated Cottonseed oil	14.0	28.3		
	Cottonseed oil	0.9	1.8		
	Salts[13]	3.6			
	Agar	3.6			
High fat				3.7	0.54
	Casein	29.0	18.6		
	Cornstarch	8.0	5.1		
	Hydrogenated cottonseed oil	51.6	74.5		
	Cottonseed oil	1.2	1.7		
	Salts[13]	5.1			
	Agar	5.1			
High carbohydrate				2.2	0.30
	Casein	17.0	17.9		
	Cornstarch	76.0	80.2		
	Cottonseed oil	0.8	1.9		
	Salts[13]	3.1			
	Agar	3.1			

[13] Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1919, **37**, 557.

Sixteen animals, taken when 28 days of age, were placed on each diet for 28 days. At the end of the experimental period, after 24 hours' fasting (except that in the first series those on the standard diet were fasted only 4 hours), one-half of each group received 3U/kg. of insulin and the others, acting as controls, were given no insulin. The pooled whole blood (collected under amytal anesthesia) from the 8 rats in each division was used to determine inorganic phosphorus by the method of Fiske and Subbarow,[7] total and total acid soluble phosphorus by a modification of the same method,

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

lipoid phosphorus by a modification of the Bloor[8] and Fiske and Subbarow methods, and the blood sugar by Somogyi's modification of the Shaffer-Hartmann method.[9] The entire procedure was repeated with a second set of rats.

TABLE II.
Growth of Rats from 28th to 56th Day of Age on the 3 Diets.

Diets	Series	No. of rats	Initial wt. gm.	Total gains gm.	Total food intake gm.	Food intake per gm. gain, gm.	Calories per gm. gain
Standard	1	16	58	106	274	2.6	11.1
	2	16	62	140	330	2.4	
High fat	1	16	52	92	176	1.9	11.5
	2	16	61	106	190	1.8	
High carbohydrate	1	16	57	103	316	3.0	11.7
	2	16	62	101	316	3.1	

As can be seen in Table II, the food intake per gram gain in weight varies with the caloric content of the diet. Thus the animals ate almost exactly the same number of calories for each gram of gain in weight, in accord with the statement of Smith and Carey[10] that, within narrow limits, consumption of food by the rat is adjusted to caloric value of the ration. Moreover the amount of gain on all the diets was quite similar. These results, therefore, support the conclusions of Levine and Smith[11] that fat calories are as efficient as carbohydrate calories for the promotion of normal growth in the rat.

The results of the blood analysis are given in Table III and interestingly enough, in this experiment, the drop in inorganic blood phosphorus does not characteristically follow insulin injection but occurs only on the high fat diet, although it is found on this diet in both series. Most of the literature reporting changes in inorganic phosphate has dealt with inorganic phosphorus curves and not with a single point of phosphorus level after insulin. We arbitrarily chose one-half hour after administration of insulin as the time for taking the blood samples, since Barbour, Chaikoff, MacLeod, and Orr[12] have shown that there is a marked lowering of blood sugar within this period in the rat, and we have also found this to be the

[8] Bloor, W. R., *J. Biol. Chem.*, 1918, **36**, 33.

[9] Somogyi, M., *J. Biol. Chem.*, 1926, **70**, 599; 1930, **86**, 655.

[10] Smith, A. H., and Carey, E., *J. Biol. Chem.*, 1923, **58**, 425.

[11] Levine, H., and Smith, A. H., *J. Biol. Chem.*, 1927, **72**, 223.

[12] Barbour, A. D., Chaikoff, I. L., MacLeod, J. J. R., and Orr, M. D., *Am. J. Physiol.*, 1927, **80**, 243.

case. It is possible, however that at this time the inorganic phosphate had not yet approached its lowest level. If, in our study, within one-half hour after insulin injection, the inorganic blood phosphorus was just starting on the downward slope (and in all but one case, where there was no change, the trend after insulin was slightly in favor of a decrease) it would seem that a high fat diet increases the sensitivity of the inorganic phosphorus to insulin activity.

TABLE III.
Blood Sugar and Blood Phosphorus Partition in mg. % on the 3 Diets*

Blood Constituent	Series	Standard Diet No Insulin	Standard Diet Insulin	High Fat Diet No Insulin	High Fat Diet Insulin	High Carbohydrate Diet No Insulin	High Carbohydrate Diet Insulin
Sugar	1	139	38	99	37	83	24
	2	79	lost	88	21	74	—
Inorganic Phosphorus	1	6.3	6.0	6.1	4.3	6.0	5.9
	2	6.6	6.6	6.0	5.4	6.8	6.7
Total Acid Soluble Phosphorus	1	37.8	39.2	40.7	38.8	41.5	42.7
	2	39.9	41.3	40.7	41.3	42.8	43.9
Lipoid Phosphorus	1	12.7	11.3	11.5	10.3	10.3	11.3
	2	10.7	10.3	11.2	10.1	10.7	11.2
Total Phosphorus	1	51.5	50.9	51.8	50.4	52.4	53.8
	2	50.8	51.9	52.4	51.7	53.8	55.4
Difference of Total Acid Soluble and Inorganic Phosphorus (Calculated Organic Phosphorus)	1	31.5	33.2	34.6	34.5	35.5	36.8
	2	33.3	34.7	34.7	35.9	36.0	37.2

*Each figure represents the results of 2 to 4 duplicate determinations on the pooled whole bloods of 4 male and 4 female rats.

Since this is apparently the first attempt to observe changes in the inorganic blood phosphorus after insulin in the rat, the effect noted may be explicable as due to species differences in this matter between the rat and the rabbit, dog, and human subjects hitherto studied.

The total acid soluble and lipoid fraction are fairly constant on the 3 diets with and without insulin, slight changes in one partition being balanced by compensatory changes in another, so that the total phosphorus remains practically unchanged. The high carbohydrate diet is an exception, since there is some evidence of an increase in the total phosphorus on this diet following insulin administration. In any case, however, there does not appear to be a loss of phosphorus from the blood as suspected by other workers.

The calculated organic phosphorus figures are interesting in view of the controversy in the literature concerning the effect of insulin on this fraction. The changes that we found, an increase in the organic partition of about 3% in over 80% of the cases following insulin injection, are not of sufficient magnitude to warrant definite conclusions, but appear to indicate that there is a tendency, under the conditions of this experiment, for the blood organic phosphorus to increase during insulin activity. Such a change is in contrast with the results of Kerr[5] but in agreement with those of Briggs, Koechig, Doisy, and Weber[2] and those of Kay and Robison.[4]

Conclusions. It may be concluded, then, that after insulin injection, (a) the blood inorganic phosphorus of rats decreases only on the high fat diet, (b) the other determined phosphorus fractions vary only slightly, (c) there does not appear to be a loss of phosphorus from the blood, and (d) there is a tendency for the organic phosphorus of the blood to increase.

8210 C

Intermediate Hosts of Aelurostrongylus Abstrusus of the Cat.

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The conception of the life history of the lungworm *Aelurostrongylus abstrusus* of the cat is based upon the work of Cameron as set forth in a series of papers by this author. No information, however, in regard to the development of intermediate forms of the first stage larvae in the supposed intermediate host, the mouse, is available from his studies.

Necropsies of 500 housecats of the city of San Francisco revealed the presence of *Aelurostrongylus abstrusus* in 8 instances. Twenty-two autopsies performed on cats from 20 other localities in California added 2 more cases. This material has been used in the present study.

The first stage larvae of *Ael. abstr.* behave in a manner usual among larvae of Synthetocaulinae known at the present time, according to our observations. Fry and Stewart[1] experimenting with

[1] Fry, W., and Stewart, T. Th., *Parasitology*, 1932, **18**, 34.