teristics. However, the data show that individual mouse strains may differ one from another in plaque size, sensitivity to agar inhibitors and hemagglutination properties.

The diameter of the plaques formed by the mouse adapted strains increased slightly when DEAE-dextran was incorporated in the agar overlay. Since the concentration of DEAE-dextrose was sufficient to allow the inhibitor sensitive r^+ variant to attain a size comparable to inhibitor insensitive r, it seems likely that the sulfated polysaccharide inhibitor in agar is only one of the factors affecting the size of plaques. The data of Ellem and Colter using their strain of Mengo and L cells overlaid with methyl cellulose support this conclusion(11).

Summary. Comparative studies were carried out using 5 strains of virus known by the designations EMC, COL SK and Mengo. Hemagglutination-inhibition tests showed that the strains were antigenically similar although not necessarily identical. Each was highly neurotropic and produced myocardial damage in mice. Plaques produced by the 5 virus strains in L cell monolayers differed morphologically. The size of the plaques formed by 3 mouse-adapted strains was roughly correlated with sensitivity to an inhibitor in extracts of agar and increased when DEAE-dextran was incorporated in the overlay. However, evidence is presented which indicates that factors other than inhibitor sensitivity also contribute to differences in plaque size. Each of the strains hemagglutinated erythrocytes of 4 mammalian species. Hemagglutination by some strains was temperature dependent, whereas with others it was not.

1. Takemoto, K. K., Liebhaber, H., Virology, 1961, v14, 456.
2. Helwig, F., Schmidt, E. C. H., Science, 1945, v102, 31.
3. Dick, G. W. A., Smithburn, K. C., Haddow, A. J., Brit. J. Exp. Path., 1948, v29, 547.
4. Jungeblut, C. W., Sanders, M., Proc. Soc. Exp. Biol. & Med., 1940, v44, 375.
5. Murnane, T. G., Craighead, J. E., Mondragon, H., Shelokov, A., Science, 1960, v131, 498.
6. Craighead, J. E., Shelokov, A., Proc. Soc. Exp. Biol. and Med., 1961, v108, 823.
7. Liebhaber, H., Takemoto, K. K., Virology, 1961, v14, 502.
8. Dick, G. W. A., J. Immunol., 1949, v62, 375.
9. Warren, J., Smadel, J. E., Russ, S. B., ibid., 1949, v62, 387.
10. Furusawa, E., Cutting, W., Proc. Soc. Exp. Biol. and Med., 1962, v109, 417.
11. Ellem, K. A. O., Colter, J. S., Virology, 1961, v15, 340.

Received February 18, 1965. P.S.E.B.M., 1965, v119.

Serum Alkaline Phosphatase in the Post-Natal Pig and Effect of Serum Storage on Enzyme Activity.* (30197)

C. H. LONG,† D. E. ULLREY AND E. R. MILLER (Introduced by W. H. Pfander)
Department of Animal Husbandry, Michigan State University, East Lansing

Few studies have been conducted on serum alkaline phosphatase levels in newborn pigs. According to Young and Underdahl(1), activity is highest at birth, falls considerably by the fifth day, and gradually declines to 50 days post-partum. The effect of prolonged storage on alkaline phosphatase in swine serum has apparently not been reported.

The objectives of this study were (a) to determine serum alkaline phosphatase activity in the pig at intervals from birth to 4 weeks of age, (b) to relate this enzyme activity to serum protein concentration and body weight changes, and (c) to determine whether prolonged 5° or —20°C storage affects serum alkaline phosphatase activity.

Methods. Forty-one pigs from 2 gilts and 3 sows, which were on the same ration, were included in this investigation. The pigs were caught as they were farrowed, were weighed,

* Published with approval of Director of Michigan Agri. Exp. Station as Journal Article No. 3567.

† Present address: Dept. of Animal Husbandry, University of Missouri, Columbia.

TABLE I. Age and Serum Alkaline Phosphatase Activity.*

Litter	No. of pigs	Age: Birth	1 day	2 days	7 days	14 days	28 days
Sow 65	8	61 ± 9†	72 ±13	51 ± 7	23 ± 1§	19 ± 1§	7 ± 1 §
" 66	10	79 ± 8	46 ± 2§	37 ± 3§	22 ± 2§	17 ± 2§	7 ± .2§
" 72	10	41 ± 3	40 ± 5	70 ± 8§	12 ± 1§	—	—
Gilt 69	5	19 ± 1	30 ± 4§	24 ± 1‡	21 ± 2	—	—
" 70	8	14 ± 2	43 ± 4§	22 ± 2‡	27 ± 6§	—	—

* Micromoles of p-nitrophenol released/ml of serum/hr.
† Mean ± standard error.
‡ Significantly different from birth ($P < .05$).
§ *Idem* ($P < .01$).

a blood sample was taken from the anterior vena cava and they were returned to their mother. Parturition time averaged about 3 hours. Subsequent blood samples were taken and the pigs were weighed at 1, 2 and 7 days post-partum in all litters and at 14 and 28 days in 2 litters.

The blood samples were collected randomly from pigs of the same age and held at 20°C for 1 hour after the last pig was bled to promote syneresis. The blood was then centrifuged at 5000 *g* for 10 minutes at 5°C. The serum was removed, placed in stoppered vials and stored at 5° or —20°C. Preliminary studies indicated that there was no change in serum alkaline phosphatase activity during 6 hours of 5° storage. All analyses were performed within this period except for those analyses concerned with storage effects. The effect of storage on serum alkaline phosphatase was studied by comparing the activity of serum samples analyzed immediately after collection of the serum, after 18 hours at 5°C and at 4 weekly intervals during —20°C storage. Frozen samples were thawed by permitting them to stand at 20°C. Serum protein concentration was determined by the method of Waddell(2), and alkaline phosphatase activity was assayed by the Sigma procedure(3) based on the work of Bessey *et al*(4).

Analyses of variance and correlation coefficients were calculated according to Snedecor(5).

Results. Serum alkaline phosphatase activity at 6 ages is presented in Table I. Each item represents the mean of all analytical values within a litter. Individual values were highly variable both within and between litters. At birth the serum of pigs born of sows appeared to have higher alkaline phosphatase activity than serum of pigs born of gilts. The mean values declined between 2 and 7 days in all litters but one, and continued to decline in those litters examined to 28 days.

The mean serum alkaline phosphatase value at each age for all pigs is presented in Fig. 1. Likewise, mean serum protein concentration and average weight at each age are presented. Serum alkaline phosphatase activity was negatively correlated with weight (—.53) and with serum protein concentration (—.33).

The effect of storage on serum alkaline phosphatase activity is shown in Table II. Each value represents the mean of 3 determinations. Storage at 5°C for 18 hours resulted in an increase in activity. Storage at —20°C resulted in higher enzyme activity after one week than in the fresh sample in serum obtained at birth. Values after 2, 3

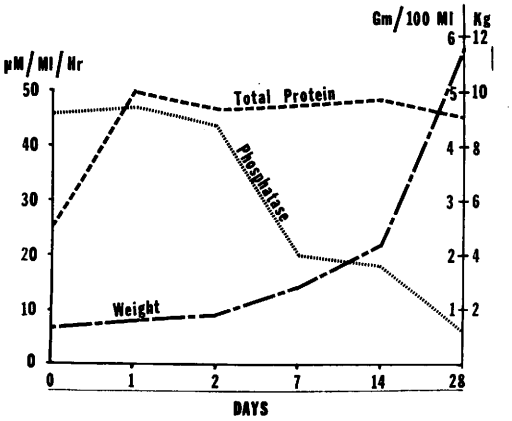

FIG. 1. Relationship between serum alkaline phosphatase activity, serum protein concentration, body weight and age.

TABLE II. Storage and Serum Alkaline Phosphatase Activity.*

Age of pigs, days	Fresh	Stored 5°C 18 hr	Stored -20°C 1 wk	2 wk	3 wk	4 wk
Birth	28 ± 1†	40 ± 1 ‡	35 ± 1‡	30 ± .2	29 ± 2	29 ± 2
1	51 ± 2	87 ± 1 ‡	49 ± 1	34 ± 1 ‡	44 ± 1‡	38 ± 3‡
7	25 ± 1	29 ± .1‡	20 ± 1‡	18 ± .1‡	17 ± 2‡	15 ± 2‡
14	17 ± 1	—	13 ± 1‡	8 ± 0 ‡	—	—

* Micromoles of p-nitrophenol released/ml of serum/hr.
† Mean of 3 samples ± standard error.
‡ Significantly different from fresh sample ($P < .01$).

or 4 weeks of —20°C storage were essentially the same as in the fresh sample. The enzyme activity of serum obtained from pigs at 1 day, and 1 and 2 weeks of age generally declined during 4 weeks of —20°C storage.

Discussion. Although appreciable variation was apparent within and between litters, serum alkaline phosphatase activity followed the declining trend after 1 day post-partum reported by Young and Underdahl(1). The most abrupt decline was observed between 2 and 7 days post-partum. The decrease at 2 days is the converse of alkaline phosphatase activity in the pig intestine reported by Payne and Marsh(6), who noted a 7-fold increase in this tissue.

The negative correlation between serum alkaline phosphatase activity and weight was statistically significant ($p<0.05$). It would appear that as body mass increased, the phosphatase available for maintenance of serum levels declined. Fletcher *et al*(7) attempted to correlate serum alkaline phosphatase activity with weight gains in cattle. The correlation coefficients obtained ranged from —.35 to .63. The cattle studied were all of greater physiological age than the pigs in this study.

The investigation of storage effects on serum alkaline phosphatase activity would suggest that enzyme assays should be completed as soon as possible after withdrawing the blood sample. Although other workers have indicated that freezer storage does not affect enzyme activity up to 14 days for cattle serum(8) and up to 4 weeks for chicken serum(9), significant deviations from fresh serum values in swine provides evidence that data derived after —20°C storage may not have absolute validity. It is interesting to note that values published by Combs *et al* (10), for serum taken from 1- and 7-day-old pigs and then frozen, were considerably lower than fresh serum values at the same ages in this study.

Summary. Alkaline phosphatase activity in the serum of 41 pigs was studied at 1, 2, 7, 14 and/or 28 days post-partum. Individual values were highly variable both within and between litters. Activity generally, but not always, decreased after birth. Negative values of .53 and .33 were obtained when serum phosphatase activity was correlated with body weight and serum protein concentration, respectively. Storage of serum at 5°C for 18 hours significantly increased phosphatase activity. Serum collected at 1, 7 or 14 days post-partum significantly decreased in phosphatase activity during 5 weeks of storage at —20°C.

1. Young, G. A., Underdahl, N. R., J. Biol. Chem., 1948, v172, 759.
2. Waddell, W. J., J. Lab. Clin. Med., 1956, v48, 311.
3. Sigma Technical Bull. 104, 1963, Sigma Chemical Co., St. Louis, Mo.
4. Bessey, O. A., Lowry, O. H., Brock, M. J., J. Biol. Chem., 1946, v164, 321.
5. Snedecor, G. W., Statistical Methods, 1956, 5th ed., Iowa State College Press, Ames.
6. Payne, L. C., Marsh, C. L., Fed. Proc., 1962, v21, 909.
7. Fletcher, J. L., Shrode, R. R., Kunkel, H. O., J. Animal Sci., 1956, v15, 1119.
8. Kunkel, H. O., Stokes, D. K., Anthony, W. B., Futrell, M. F., ibid., 1953, v12, 765.
9. Wilcox, F. H., J. Exp. Zool., 1963, v152, 195.
10. Combs, G. E., Wallace, H. D., Alsmeyer, W. L., Koger, M., J. Animal Sci., 1959, v18, 361.

Received February 19, 1965. P.S.E.B.M., 1965, v119.