blood pH, age of the animals, and duration of OHP treatment may influence respiration and gasping. These factors are known to be important in other aspects of hypothermic and OHP stress(1,9).

Summary. The effects of combining pressure oxygenation at 3 atmospheres with hypothermia (20°C) on asphyxial survival of rats following tracheal occlusion have been studied. Exposure to 100% oxygen at high pressure (OHP) without hypothermia increased survival times about 55-80% over those seen in animals breathing 100% oxygen at ATP. Oxygenation at 3 atmospheres combined with hypothermia at 20°C gave maximal prolongation of survival, although this was not significantly greater than seen with hypothermia alone. Addition of CO_2 to the inspired O_2 under pressure also afforded additional protection, even in absence of hypothermia. The action of OHP and hypothermia in increasing survival is additive rather than synergistic.

1. Dripps, R. D., ed., *Physiology of Induced Hypothermia,* Publ. No. 451, Nat. Acad. Sci., Nat. Res. Council, Washington, 1956.

2. Tschirgi, R. D., Gerard, R. W., *Am. J. Physiol.,* 1947, v150, 358.

3. Selle, W. A., PROC. EXP. BIOL. AND MED., 1943, v54, 291.

4. Hiestand, W. A., Tschirgi, R. D., Miller, H. R., *Am. J. Physiol.,* 1944, v142, 153.

5. Boerema, I., *Surgery,* 1961, v49, 291.

6. Marshall, J. R., Lambertsen, C. J., *J. Appl. Physiol.,* 1961, v16, 1.

7. Adolph, E. F., Naberschnig, A., Orchard, D. P., *ibid.,* 1961, v16, 819.

8. Kety, S. S., Schmidt, C. F., *J. Clin. Invest.,* 1948, v27, 484.

9. Bean, J. W., *Physiol. Rev.,* 1945, v25, 1.

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Further Evidence of a Relationship between Live Fowl Pox Virus and Parthenogenesis in Turkey Eggs. (27386)

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Data presented in 1956 indicated a possible relationship between live fowl pox and pigeon pox viruses with parthenogenesis in turkeys and chickens(1). Later, live Roux sarcoma virus was shown to be effective in enhancing an unorganized type of parthenogenetic development in turkeys(2). A relationship was found by Gill and Stone at the Animal Disease and Parasite Research Division, Agricultural Research Center, Beltsville, Md., between live Newcastle disease virus and the level of parthenogenesis in turkey eggs (personal communication). Each of these 3 live viruses, however, upon being inactivated with beta-propriolactone and subsequently used to vaccinate virgin turkey hens proved to be ineffective in increasing the level of parthenogenetic development(3). Spontaneous parthenogenesis in unfertilized White Leghorn chicken eggs also has been noted following a natural outbreak of fowl lymphomatosis of the visceral type(4).

Data presented earlier on the effectiveness of live fowl pox and pigeon pox viruses involved use of unfertilized eggs of Dark Cornish chickens and Beltsville Small White turkeys. The effects of live virus vaccines were measured by comparing the average level of parthenogenesis in eggs of the same birds before and after vaccination. This method, while keeping genetic influences constant, failed to take into consideration minor seasonal fluctuations in the level of parthenogenesis. Therefore, it was desirable to conduct additional tests with live fowl pox virus so that daily collections of eggs from 2 similar groups of birds could be incubated and examined simultaneously for parethenogenetic development.

Material and methods. These tests involved a total of 50 virgin Beltsville Small White (BSW) females from a flock in which no selection for increased incidence of parthenogenesis had been practiced. Each of these

50 females when 6-8 weeks of age had received a routine vaccination for fowl pox.

At approximately 16 weeks of age, the 50 birds were removed to isolated quarters to reduce chances of subsequent natural exposure to fowl pox infection. They were maintained on a 10-hour light day until 30 weeks of age when the light day was lengthened to 14 hours. First eggs were laid approximately 2 weeks following onset of the longer light day. Eggs, as collected, were identified with hen numbers and date of lay and each evening placed in an incubator operating at a temperature of 99¾°F and at a relative humidity of 57%. All eggs received an initial 10-day period of incubation before being broken-out and examined macroscopically for parthenogenetic development.

On the 42nd day of egg production, the hens were segregated at random into 2 groups. Twenty-three hens (hereafter the treated group) were removed to another isolated poultry house where they were revaccinated for fowl pox. The vaccine was a commercial product containing live fowl pox virus propagated on the chorio-allantoic membranes of embryonated chicken eggs. Administration was *via* the wing web by the stick method using a 2-pronged applicator.

The remaining 27 hens were not revaccinated or moved and were maintained as a control group isolated from other turkeys.

Results and discussion. Data presented in Fig. 1 show the average percentage level of parthenogenesis observed in unfertilized eggs of treated and control groups of turkeys. The data show a sharp rise in the level of parthenogenesis, evident as early as the 6th day after revaccination. Percentage values of parthenogenesis for eggs of the treated group continued at a high level and well above those of the controls throughout the 90-day test period. Differences between levels of parthenogenesis among eggs of treated and control groups, when tested by analysis of variance, were found highly significant at the one percent level.

The treated group laid a total of 1091 eggs during the 90-day test period following revaccination. Of these, 314 or 27.9% showed some degree of macroscopically observable

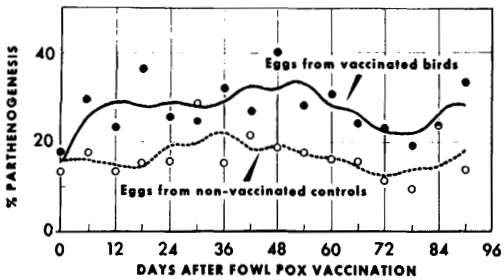

FIG. 1. Percentage parthenogenesis in unfertilized eggs of Beltsville Small White turkeys in relation to time of fowl pox vaccination. Hens of treated group received 2 vaccinations for fowl pox, the first at 6-8 wk of age, the second 30 wk later. "Non-vaccinated" controls received a routine vaccination at 6-8 wk but were not revaccinated.

parthenogenetic development. This included 41 eggs (3.9%) in which blood formation occurred and 24 other (2.7%) in which well formed embryos, as well as blood, had developed. Four sets of identical twins were encountered in these single yolked eggs following use of the vaccine.

The control group during this same 90-day test period produced a total of 1099 eggs. Of these, 188 (17.1%) showed some degree of macroscopically observable parthenogenesis. Sixteen of these eggs (1.5%) showed varying degrees of blood formation while 4 additional eggs (0.36%) were encountered in which embryos, as well as blood had developed. No twin embryos were found in eggs of this group. Thus while the level of parthenogenesis of all categories increased by something over 50%, blood formation tripled and the embryo population increased 6-fold following revaccination.

The record of both groups prior to their use in these tests may be of interest. The 50 hens were maintained as a single flock during the 42-day period prior to revaccination.

The 23 hens, later to become the treated group, produced 597 eggs during this 42-day pre-test period of which 106 or 17.8% developed parthenogenetically. This included 7 eggs (1.2%) in which blood formation occurred and 4 other eggs (0.75%) in which embryos developed.

The 27 hens which were to serve as controls laid 734 eggs during this same 42-day period, of which 100 or 13.6% were classified as having undergone some degree of partheno-

genetic development. This included 10 eggs (1.4%) in which blood only occurred and 4 other eggs (0.5%) in which well formed embryos had developed. Thus while total average incidence of parthenogenesis was slightly lower in eggs of control birds, the percentage value for eggs showing blood formation and embryo formation was essentially the same for both groups.

The nature of the stimulus to parthenogenetic development and mode of action of viruses in turkeys remain alike obscure. However, a few points concerning the enhancing action of live viruses appear to be fairly well established. These findings are based entirely on experiments with birds of the closed flock of Beltsville Small White turkeys (not selected for parthenogenesis) in which relatively advanced parthenogenetic development was first observed(5,6), and do not necessarily apply to other strains of turkeys which may not produce eggs inherently capable of parthenogenetic development(1). (1) Killed viruses are ineffective. Incidence of parthenogenesis was not increased in eggs following vaccination or revaccination with viruses inactivated with beta-propriolactone(3). (2) Antibodies as such can probably be discounted as the agents responsible for parthenogenesis. Killed Newcastle disease virus incites antibody formation and is effective in protecting chickens from this disease. Parthenogenesis, however, did not increase in turkey eggs following vaccination with killed Newcastle virus(3). (3) Foreign proteins present in the vaccine are probably not the causative agents. Virus free, macerated chicken embryos and membranes when injected subcutaneously into turkey hens did not cause an increase in incidence of parthenogenesis in their eggs(3). (4) The effective duration of a single vaccination is of the order of 3 months or longer(2), (Fig. 1). This possibly means that causative agents have gained entrance into numerous immature ovarian follicles following vaccination. The health of turkeys is not affected, the ovary remaining functionally unimpaired following vaccination. Cleaved immature ovarian eggs are found rarely. It is reasonable to assume, therefore, that the agent involved in parthenogenesis although present, remains latent until time of ovulation when a single maturing follicle comes under the influence of the luteinizing hormone.

Summary. The level of parthenogenesis was determined in unfertilized eggs of 2 similar groups of unselected Beltsville Small White turkeys. Birds of one group were revaccinated for fowl pox (live virus) at approximately 36 weeks of age. The others serving as controls had received only one vaccination when 6-8 weeks of age. The treated group produced 1091 eggs and the controls 1099 eggs during the 90-day test period. Approximately 28% of the eggs from the treated group and about 17% of the control group underwent some degree of parthenogenetic development during 10 days of incubation. Twenty-four embryos (2.7%) were encountered in eggs of the treated hens as compared to 4 (0.36%) in eggs of the controls.

1. Olsen, M. W., *Science,* 1956, v124, 1078.
2. ———, *Nature,* 1961, v190, 191.
3. ———, *Am. J. Vet. Research,* in press.
4. ———, unpublished observations.
5. Olsen, M. W., Marsden, S. J., PROC. SOC. EXP. BIOL. AND MED., 1953, v82, 638.
6. Olsen, M. W., *Science,* 1960, v132, 1661.

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