basement membrane at the epithelio-connective tissue junction, b) precocious decay of the tonofibrils in the stratum spinosum, and c) parakeratosis of the surface layers. a) Absence of a distinct border structure between epithelium and supporting connective tissue could indicate that one point of attack of vit. A deficiency possibly lies in the connective tissue. The basement membrane belongs to the ground substance of connective tissue and encloses a network of reticular fibers(2). The connection of this reticulum with the tonofibrils of the epithelium is altered in the mucosa of vit. A deficient rats. At various places connective tissue penetrates the epithelial sheet, limiting the latter to one layer of living cells. This points to a block in the trophic supply of the epithelium by the connective tissue in vit. A deficiency. The role of vit. A in the metabolism of connective tissue obviously warrants histochemical studies. b) Precocious, granular involution of the tonofibrils is related to the well-described metabolic dysfunction of epithelium in vit. A deficiency(3). Loss of tonofibrillar continuity within cells with a nucleus constitutes a specific disturbance that is not a stage of the normal cytomorphic changes of oral rat epithelium. Apparently it is identical with the histopathologic condition termed spongiosis. These observations on undenatured oral epithelium throw some new light on the nature of the prominent, but little known, protoplasmatic cell structures called tonofibrils. They corroborate the views previously expressed(1) that the presence of tonofibrils is bound to the vitality of the cell. Since vit. A, probably through intercession of connective tissue, controls differentiation and longevity of the epithelium, it directly or indirectly secures maintenance of tonofibrillar continuity. c) Keratinizing metaplasia, usually described as the main effect of vit. A deficiency, is a physiologic condition in oral rat epithelium. However, this keratinizing process is also disturbed in vit. A deficiency. Due to precocious decay of tonofibrils the surface layers are only slightly cohesive and very irregular in width.

Summary. Biostructures of oral epithelium such as tonofibrils and basement membrane were ascertained in normal rats with the help of phase contrast microscopy. Vit. A deficiency effected in oral rat epithelium 1) aplasia of the basement membrane, 2) precocious disintegration of the tonofibrils in the stratum spinosum, and 3) parakeratosis of the surface layer.

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Cooling of Embryonated Eggs to Produce an LD_{50} for *Coccidioides immitis*. (20359)

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(Introduced by Charles M. Carpenter.)
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In studying a chronic disease process, an investigator is faced with the problem of choosing an objective criterion that is suitable for the evaluation of the progress of the disease or the effect of a therapeutic procedure. Procedures that utilize the evaluation of the LD_{50} are frequently used. However, agents that produce chronic diseases usually do not kill a large percentage of infected individuals in a relatively short period of time. Therefore, especially susceptible host species must be used or some other modifying procedure employed. Frequently physical agents such as X-ray(1) or chemical substances such as

cortisone(2) have been used to decrease the resistance of the host and thus improve the probability of a successful invasion by the infective agent.

This laboratory has been studying *Coccidioides immitis*. This fungus when it disseminates in man produces clinical symptoms very similar to tuberculosis. It is highly infectious and can be established easily in experimental animals. The degree of involvement, however, is highly variable between individual animals. For example, identical inoculations of organisms into 10 rabbits of the same strain and age produced the following results: Two animals died of the infection in 6 weeks; 3 were chronically ill for 4 months and longer; 3 remained apparently healthy but at autopsy showed numerous widely disseminated lesions; and 2 showed no symptoms and no lesions at autopsy. Guinea pigs were more consistent in establishing the infection but varied considerably as to survival and clinical state of the infection. *Massive doses* of as many as one million arthrospores seldom produced death in eggs when inoculated into the allantoic fluid, although the mycelial stage of the organism was frequently observed on the chorion-allantoic membrane. When such massive doses do not cause death, an LD_{50} can be attained only by increasing the virulence of the invading organism, increasing the susceptibility of the host, or by using another host. Chicken embryos are particularly desirable hosts as they are generally initially sterile, are easily housed and maintained, create no diet problems, can be handled in statistically significant numbers, and are relatively inexpensive.

Methods. For these reasons it was decided to try to attenuate the chick embryos so that their resistance to the invading organism would be lowered and the LD_{50} thus attained. Although agents such as X-ray(1) and toxic chemicals(2) have been used, the simple procedure of cooling the eggs on their 9th day of incubation was employed. Eggs that have been incubated 8 days can be left in the disconnected incubator for 24 hours and still hatch without apparent ill effects on the 21st and 22nd day. The incubator in this laboratory was so located in the building that it cooled in 24 hours to 22°-24°C, as indicated by a maximum-minimum thermometer. The entire procedure consisted of incubating fertile eggs for 8 days at 38.5°C. After the viable eggs were allowed to cool to room temperature circa 26°-27°C, they were drilled, inoculated with *Coccidioides immitis* suspensions, and sealed. The *Coccidioides immitis* suspensions were harvested from mycelial growths that had developed arthrospores on Sabouraud dextrose agar. These slants were wetted with 5% glucose in saline solution. Great care was taken to minimize possible chance infection of personnel. After adequate material had floated free, the fluid was transferred to a sterile bottle containing glass beads. This was placed in a shaker to disperse the organisms further. This suspension was then tested for the number of viable spores by serially "diluting out" in liquid media and also by plating on Sabouraud dextrose agar. These counts were used to calculate the number of viable organisms in the suspensions used for inoculation of the eggs. When the egg incubator had cooled to about room temperature the eggs were returned and allowed to remain in the closed incubator until the following morning. The incubator was then turned on and the eggs candled. The eggs were candled each morning and the number of survivals noted. To minimize the danger of infecting personnel, all surviving infected eggs were killed just before hatching.

Results. Table I shows the number of cooled embryos that survived graded inoculations of *Coccidioides immitis*. LD_{50} dosage, calculated by the Reed-Muench formula(3), was 3500 spores, whereas 3 separate doses of 1×10^6 spores each on 3 different days in embryos that were not cooled produced no significant fatalities.

There were no significant fatalities of cooled embryos from the inoculation with autoclave-killed (½ hr-15 lb) suspensions of *Coccidioides immitis*.

Conclusion. 1. It is clear that the cooling of chick embryos on the 9th day has made it possible for the invading organisms to increase their effectiveness in overcoming the host. This technic has the advantage of a flexible and easily controlled attenuating device. The

TABLE I. Survival of 8-Day Eggs, Cooled 24 Hours, Inoculated with 0.1 cc Suspensions of *Coccidioides immitis*.

No. of 8-day eggs	Dosage	Days of survival in total age of embryo							
		9	10	11	12	13	14	15	16
46	1×10^6 spores	45	12	5	1	0	0	0	0
47	1×10^5	46	27	15	10	8	7	4	4
47	1×10^4	46	41	37	33	27	24	23	23
47	1×10^3	45	41	38	37	33	29	26	23
45	0.1 cc 5% glucose in saline	44	42	41	40	40	38	38	38

length of time and temperature of cooling can be chosen so that various degrees of susceptibility might possibly be attained if other invading hosts were to be used. 2. However, a word of caution is needed if chick embryos are to be used in a pseudo-quantitative manner to evaluate virulence or invasive capacity of an infective agent. Considerable variation on the hatchability or vigor of the embryos may occur from one region or one strain of chickens to another(4). Also nutritional factors of the mother hen, her age, and the phase of the egg laying season(5-8), all enter into the percentage of eggs that will hatch; hence, reflect the possibility of inherited variation of the susceptibility of the host.

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Proteolytic Activity and Protein Catabolism of Rat Diaphragm in Hemorrhagic Shock.* (20360)

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This work forms part of a study concerned with the biologic role of certain intracellular proteolytic enzymes. These peptidases, intensively and extensively studied by Bergmann and associates(1), exhibit a high degree of specificity so that it has been possible to measure accurately enzyme activity of highly inhomogenous preparations as a function of the rate at which, under standard conditions, a specific synthetic peptide substrate is hydrolyzed. Thus, glycylglycine‡ (GG) is hydrolyzed by a dipeptidase (GGase), present in many tissues, which has been shown by Smith(2,3) to be quite specific in its cleavage of this particular dipeptide.

That a state of shock serves as a potent stimulus to protein catabolic processes appears to be well documented. A review of these and other metabolic changes has been presented recently by Engel(4). The present investigation was undertaken to determine whether shock-induced acceleration of protein

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