of this initial damage are not manifested to the same degree as in the normal animal. The marked lowering of the metabolic rate during hibernation is apparently responsible for the prolongation of the survival time in the irradiated, hibernating animals either through postponing the tissue damage or by markedly slowing the rate of disruption of the cellular metabolic processes by x-ray. Studies by Smith and Grenan(3) have indicated that at least some of the blood changes characteristically seen after x-ray, do not occur while irradiated woodchucks and marmots are in the hibernating state. Petersen and DuBois (6) found that lethal doses of x-ray produce an increase in the activity of alkaline phosphatases of the spleens of ground squirrels. The amount of increase in enzyme activity was less in hibernating, irradiated animals than in non-hibernating animals receiving the same dose of x-ray.

The results of this investigation showed that ground squirrels irradiated while in the homeothermic state and maintained thereafter at 5°C failed to exhibit any significant prolongation of survival time. It seems likely that the inability of irradiated ground squirrels to undergo hibernation with a consequent decrease in metabolic rate accounts for this lack of protection. It has been demonstrated that amphibians(5) kept at low temperatures after x-irradiation exhibit a prolonged survival time. Thus if it were possible to induce hibernation and the coincident lowering of the metabolic rate immediately following irradiation, a protective effect might be noted in ground squirrels irradiated while in the non-hibernating state. Extension of the results of this investigation may provide further insight into the mechanism of radiation lethality.

Summary. The LD_{50} of x-ray for non-hibernating ground squirrels (*Citellus tridecemlineatus*) is approximately 700 r. Non-hibernating animals given 800 r of x-ray exhibited over 80% mortality within 10 days. Ground squirrels irradiated while in hibernation and maintained in the hibernating state following irradiation exhibited no mortality within 30 days following exposure to x-ray, but when these animals were returned to the homeothermic state either at 2 weeks or at 4 weeks after irradiation, death ensued within 10 days. Animals which were maintained in hibernation following lethal doses of x-ray eventually succumbed, although survival was greatly prolonged. Thus, while hibernation markedly increased the survival time in this species following lethal doses of x-ray, it did not alter the ultimate mortality.

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A Growth Factor Required by *Donovania granulomatis.**† (20648)

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Little is known concerning the exact requirement for growth of the Donovan body (*Donovania granulomatis*). Anderson(1) reported the cultivation of the Donovan body

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in the yolk sac of the chick embryo, and later (2), on the embryonic yolk alone. Dunham and Rake(3) and Rake and Oskay(4) were able to grow the Donovan body on slants consisting of tryptose-beef heart infusion broth, Leventhal's stock broth and agar but did not obtain luxuriant growth. Dienst and associates(5) developed a medium containing fresh egg yolk, Bacto-peptone, Bacto-tryptone, dextrose, sea salt and agar in which luxuriant growth of the Donovan body was obtained. In a recent investigation of the bacteriological behavior of *D. granulomatis*, Goldberg, Weaver and Packer(6) studied the unknown factor found in fresh egg yolk required by the organism for growth. The factor varied in amount in eggs from different sources and seemed to be most abundant in eggs from chickens on a "natural diet," although it was also found in infertile duck, goose and turkey eggs. These investigators were able to extract with Locke's solution from chicken egg yolk (coagulated at 80-85°C for 10 minutes) an active material which gave a positive biuret and Molisch test and was not filterable through an ultrafilter. None of the following substances would replace egg yolk to support growth of the Donovan body: rabbit liver extract, grass extract, 1% yeast extract (Difco), rabbit liver extract with 1% gastric mucin, thiamin hydrochloride, cystine, asparagine, biotin, pimelic acid and a synthetic medium containing casamino acids, glucose, cystine, tryptophan, asparagine, uracil, adenine, guanine, xanthine, pantothenic acid, riboflavin, nicotinic acid, biotin, pyridoxine, folic acid, thiamin hydrochloride and p-aminobenzoic acid. On comparison of the morphology of the Anderson and Dienst strains of *D. granulomatis*, Goldberg and associates found differences in morphology resulted from differences in the media used by these investigators.

The present paper is concerned with a study of the factors found in egg yolk which are necessary for the growth of the Donovan body.

Experimental. In general, all procedures used in this study were repeated 3 or more times with consistent results. The eggs used were day-old eggs from a reliable source which supplied eggs with a high content of the growth factor. *Assay procedure.* The preparation to be tested is dissolved or suspended in sufficient fluid to make 100 ml and then is added in place of the diluted egg yolk to the medium of Dienst *et al.*(5).‡ The pH of the medium is adjusted to between 7.2 and 7.4 and the preparation is sterilized by autoclaving at 15 lb pressure for 15 minutes. Two of the tubes containing 5 ml each of the medium to be tested are inoculated with 1/10 ml from a 2-day-old culture of *D. granulomatis* and then are incubated for 2 days before being similarly transferred to new tubes of the same medium. At the time each transfer is made, the extent of growth is determined by examining a stained smear made from a loopful of the culture. If the organism will grow luxuriantly on the test medium through 4 or 5 transfers, this is considered to indicate an adequate level of the growth factor in the medium. The cultures of the Donovan body used in these studies were the A strain and Z strain isolated in 1947 from active lesions of patients suffering from granuloma venereum and have been subcultured since that time on the medium of Dienst and associates(5). The stock cultures are kept at incubator temperature and transferred 3 times per week. This assay procedure proved to be adequate to show gross differences in distribution and activity for various preparations and possible sources of the growth factor, although the procedure was modified at a later date to give results in terms of arbitrary "units of activity."

‡ Bacto peptone 0.3 g, Bacto tryptone 0.3 g, glucose 0.3 g, sea salt or crude rock salt 0.2 g and agar 0.12 g. This is dissolved in 100 ml water and the pH is adjusted to 7.3. After autoclaving, the yolks from 2 fresh eggs are removed aseptically, diluted with 50 ml of sterile saline and added to the medium. The approximately 200 ml of medium resulting is then measured aseptically into culture tubes (5-6 ml to a tube). The tubes are incubated for 30 minutes in a water bath at 56°C after which they are ready for use. The portion of the medium other than the diluted yolk was for convenience termed "the basal medium" and the material to be tested was added to this in place of the egg yolk. If the volume of the substance to be tested was likely to be high, a double strength basal medium was prepared to allow for greater dilution by the test substance.

Heat stability. Growth was adequate on the Dienst medium which had been autoclaved at 20 lb for 30 minutes though this resulted in coagulation of the egg yolk proteins and thus changed the physical properties of the medium from that of the medium as originally used. This and other studies of heat stability indicated that autoclaving at 15 lb pressure for 15 minutes as done during the assay procedure did not result in a significant loss of the growth factor. This is in contrast to the results of Goldberg *et al.*(6) who observed a greater degree of thermal instability in the Dulaney medium (consisting of egg yolk and Locke's solution) then was noted with the more complex Dienst medium used in the present study.

Fractionation of egg yolk. a) Dialyzable constituents. Yolks from 2 eggs diluted with an equal volume of saline was dialyzed for 36 hours through a collodion membrane or cellophane against distilled water, saline or the basal medium. Neither the aqueous or saline dialysate added to the basal medium nor the basal medium dialyzed against the yolk supported growth. *b) A protein-free aqueous extract of egg yolk.* The yolks of 10 eggs were diluted with distilled water to make a volume of one liter. This was heated to boiling with constant stirring to coagulate the protein. On filtering with the aid of Filter-Cel, a clear filtrate was obtained which was concentrated to a small volume at a low temperature by vacuum distillation. In another similar experiment no Filter-Cel was used and suspended lipids were removed from the filtrate by extraction with petroleum ether. On assay, these protein-free, aqueous extracts of egg yolk failed to support growth of the Donovan body. *c) Protein and lipid fractions from egg yolk.* The yolks of 4 eggs were added with vigorous stirring to 200 ml of a mixture of 2 parts of alcohol with one part of ethyl ether at a temperature of 2°C. After twenty minutes the mixture was filtered and the precipitate was returned to the beaker. One hundred ml more of alcohol-ether mixture was added with stirring to the precipitate and after 15 minutes the mixture was again filtered. After 3 more such washings, the precipitate was placed in a vacuum desiccator over $CaCl_2$ to remove the last traces of ether. The alcohol-ether mixture used in the process was evaporated to dryness and the last traces of ether removed in a similar fashion, since small amounts of ethyl ether inhibit growth of the Donovan body. The lipid fraction failed to support growth of the Donovan body while the medium containing the precipitated protein fraction produced luxuriant growth. The dried protein fraction still retained the growth factor after 3 months at room temperature. After a year such a preparation was no longer active. Long continued extraction of the protein fraction in a Soxhlet extractor with successive portions of alcohol, ethyl ether, and petroleum ether did not effect the growth-promoting activity of the protein fraction. *d) Separation of yolk proteins.* Lipovitellin, vitellin and lipovitellinin were separated from egg yolk as described by Alderton and Fevold(7) and Fevold and Lausten(8), except that a Spinco preparative model ultracentrifuge was used rather than the Sharples super centrifuge. In the preparation of phosvitin the procedure of Mecham and Olcott(9) was used. The other proteins of the yolk were not isolated but were tested in fractions removed in the process of isolating the proteins mentioned above. The vitellin preparation contained the major proportion of the activity of the yolk. The other fractions either were inactive, or of comparatively low activity.

Effects of hydrolysis upon the growth factor. a) Acid hydrolysis. An active nearly lipid-free preparation of the total proteins of egg yolk was hydrolyzed with 5 N sulfuric acid by heating in an autoclave for 16 hours at 15 lb pressure. The sulfate was precipitated with barium hydroxide and the filtrate concentrated to a small volume. This preparation proved to be inactive. Milder hydrolysis of egg yolk protein by autoclaving with 1 N HCl for one hour similarly destroyed the growth promoting activity of the yolk; *b) Alkaline hydrolysis* of the yolk with 1 N NaOH for one hour also destroyed the activity of the yolk; *c) Enzymatic hydrolysis.* Egg yolk was aseptically added to a sterile trypsin solution. Incubation at 37° for 2 days resulted in a loss of activity.

Other possible sources of the growth factor. A number of other possible sources of the

growth factor were tested including beef serum, chicken liver and various commercial liver extracts, milk, casein, dried whey, whole dried yeast and yeast extracts, and spinach. A mixture of vitamins and amino acids§ was tried in total and in part with various combinations and at different levels. None of these supported growth.

Summary. The studies described indicate that the growth factor in egg yolk required by *Donovania granulomatis* is non-diffusible and was destroyed by hydrolysis with acid, alkali or a crude trypsin preparation. The activity was found in the protein fraction of the yolk, and on separation of the yolk proteins, the major portion of the activity was associated with the vitellin. The factor required for growth does not seem to be any one of a number of well known growth factors which were tested, and furthermore, several other materials considered to be good sources of growth factors when used to replace egg yolk in the medium would not support growth of *D. granulomatis*.

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§ The mixture of growth factors contained: adenine 1 mg, guanine 1 mg, uracil 1 mg, xanthine 1 mg, asparagine 1 mg, nicotinic acid 10 μg, inositol 10 μg, p-aminobenzoic acid 10 μg, riboflavin 40 μg, thiamin hydrochloride 40 μg, calcium pantothenate 40 μg, pyridoxin hydrochloride 40 μg, biotin 0.1 μg, folic acid 0.1, μg, L-histidine monohydrochloride 0.3 g, L-lysine monohydrochloride 0.6 g, DL-tryptophan 0.35 g, DL-phenylalanine 1 g, DL-threonine 0.5 g, DL-methionine 0.5 g, L-leucine 0.5 g, DL-isoleucine 0.6 g, DL-valine 0.5 g and L-arginine monohydrochloride 0.8 g.

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Adrenal Function Tests in Mice Sensitized to Histamine with *Hemophilus pertussis* Vaccine.* (20649)

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Both adrenalectomized mice(1) and mice inoculated with *Hemophilus pertussis* vaccine(2) are more susceptible than normal animals to the lethal effects of histamine, of certain bacterial vaccines, and, of anaphylactic shock. Pertussis-inoculated mice can be protected by cortisone from the fatal effects of subsequent injections of histamine or pertussis vaccine(3). Cortisone likewise restores the resistance of adrenalectomized animals to histamine(4). In view of the similar responses of pertussis-inoculated and adrenalectomized mice to the materials mentioned, it seemed possible that pertussis vaccine might have an injurious effect on adrenal function. The measurement of adrenal function in mice by direct assay of the hormone in body fluids is not feasible. However, indirect indices of adrenal function may be employed. Winters, Schultz, and Krehl(5) measured liver glycogen deposition in fasting rats after ACTH as

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