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*.

Elizabeth Martin and Agnes C. Redfearn gave technical assistance in this study.

§ 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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an index of adrenal function. However, in our experiments with both normal and pertussis-inoculated mice, it was found that this method gave variable results. In one experiment, ACTH would cause a significant rise in liver glycogen; the following day, the same dose of ACTH injected in a similar group of animals would have no observable effect on liver glycogen. Much more consistent results were obtained by a procedure which tested the ability of mice in the fasting state to maintain their liver glycogen at reduced atmospheric pressures. Evans has demonstrated that rats fasted for 24 hours at ½ atmospheric pressure do not lose their liver glycogen store as do normal fasted animals (6). Liberation of adrenal steroids was shown to be essential for this endogenous carbohydrate formation (7).

Materials and methods. Adult male white mice weighing 17 to 23 g were employed. The animals were bred in our laboratory and originated from a strain purchased from Tumblebrook Farms. A group of mice was injected with 0.2, 0.1, and 0.1 ml of *H. pertussis* vaccine† on 3 successive days. Six days after the last injection, 4 of the pertussis-inoculated mice and 4 uninoculated animals were challenged intraperitoneally with 50 mg/kg of histamine dihydrochloride. If all pertussis-injected mice died and all control uninoculated mice survived, the remaining pertussis-injected animals were considered to be histamine-sensitive. The LD_{50} of histamine dihydrochloride in pertussis-inoculated mice was found to be 4.7 mg/kg, and, in uninoculated mice, 570 mg/kg. Histamine-sensitive and histamine-resistant mice were placed in separate compartments in a glass desiccator. Air was removed by a vacuum pump and the desired pressure in the chamber was obtained by adjusting an air intake valve. Experiments were run at either 380 or 278 mm Hg. At 380 mm Hg, the ventilation through the chamber was 7.9 liters per minute; at a pressure of 278 mm Hg, 5.1 liters per minute. Experiments were performed at room temperature, which varied from 60° to 70°F. Pressures were reduced gradually over a 30-minute period. No food or water was available to the animals during their 24-hour period under reduced pressure. At the end of this time, atmospheric pressure was restored, the mice were taken from the chamber, and their livers were removed under sodium pentobarbital anesthesia. The organs were then weighed, hydrolysed with 30% KOH and analyzed for glycogen by the method of Seifter *et al.*(8). Controls consisted of mice fasted at atmospheric pressure for 24 hours as well as mice at atmospheric pressure which had been fed.

† Contained 50 billion organisms/ml. Provided by Sharpe & Dohme, Inc.

TABLE I. Effect of Pertussis Vaccine Inoculation and Low Atmospheric Pressure on Liver Glycogen of Mice.

Group	Mice (No.)	Pressure (mm Hg)	Liver glycogen* (mg %)
I Uninoculated mice			
a Uninoculated fed	11	A†	1881±357
" fasted	5	"	106± 29
" "	5	380	1272± 75‡
b " fed	5	A†	2212±521
" fasted	5	"	196± 48
" "	6	278	2442±330‡
II Pertussis-injected animals			
Pertussis-inoc. fed	6	A†	2215±300
" " fasted	5	"	380± 98
Idem	5	380	1256±167‡
"	6	278	Died

* Data expressed as mean with S.E.
† A = Atmospheric.
‡ Indicates statistical significance (p=.01) when compared with respective fasted controls at atmospheric pressure.

Results. The data in Table I indicate that at an air pressure of 380 (oxygen tension of 76 mm Hg) both pertussis-inoculated and uninoculated mice in the fasting state maintained liver glycogen levels (1256 ± 167 mg % and 1272 ± 75 mg %) significantly greater than the levels (380 ± 98 mg % and 106 ± 29 mg %) of fasted controls at atmospheric pressures. At 278 mm Hg the liver glycogen of uninoculated fasted animals was 2442 ± 330 mg % as compared with 2212 ± 521 mg % for uninoculated fed animals at atmospheric pressure and 196 ± 48 mg % for uninoculated fasted animals at atmospheric pressure. Although the glycogen values of uninoculated fasted animals kept for 24 hours at 278 mm Hg reached levels com-

parable with fed animals at atmospheric pressure, 6 of 6 pertussis-inoculated mice did not survive 24 hours at 278 mm Hg, the majority of animals succumbing within a few hours after exposure.

In 2 succeeding experiments in which equal numbers of pertussis-inoculated and uninoculated mice were placed in the low pressure chamber at 278 mm Hg for 24 hours similar results were obtained. It was found that 17 of 18 uninoculated mice survived a pressure of 278 mm Hg for 24 hours. Only 3 of 18 pertussis-injected animals remained alive under these conditions. The difference between these groups is a significant one ($p = <.01$).

Discussion. It is apparent from these experiments that under moderate anoxic conditions (380 mm Hg) the adrenals of pertussis-injected, fasted mice function as well as those of uninoculated fasted animals with respect to the maintenance of liver glycogen above the fasting level of control mice. However, under more severe anoxic conditions (278 mm Hg) the survival ability of the pertussis-inoculated mouse, like that of an adrenalectomized animal(1), is low.

Although cortical extract will restore the lowered susceptibility of the adrenalectomized animal to the lethal effects of reduced oxygen tension(1), experiments employing ACTH, cortisone and aqueous adrenal extracts in order to increase the resistance of pertussis-inoculated mice to anoxia have thus far been unsuccessful.

Summary. An increase in liver glycogen occurred in both fasting uninoculated and fasting pertussis-inoculated mice when exposed to ½ atmosphere pressure for 24 hours. Exposure at ⅜ atmosphere for 24 hours killed 17 of 18 pertussis-inoculated mice and only 3 of 18 uninoculated animals.

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Prevention of Toxic Effects of Injected Thromboplastin by "Thromboplastinase".* (20650)

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Intravenous injection of thromboplastic suspensions in experimental animals has been reported to cause ataxia, convulsions and death(1,2). These effects have been prevented by prior injection of several agents such as rabbit serum, heparin, and congo red(4), soybean trypsin inhibitor(5), monosodium alpha tocopherol phosphate, and inositol phosphatide(6). The morbid or lethal effects of injected thromboplastin have been attributed to thromboembolism(1-3).

Our laboratory recently reported on a new bacterial enzyme, "thromboplastinase" which caused a marked reduction of thromboplastic potency of tissue suspensions *in vitro.* Its preparation, properties, and mode of action have been described(7). In this paper we wish to report the effect of "thromboplastinase" on the toxicity of injected thromboplastic suspensions.

Materials and methods. Active, stable, reproducible rabbit brain or human brain thromboplastic suspensions(8) were used except when indicated. The saline extract of acetone-dried tissue contained finely divided, suspended material. Such suspensions were variously treated before injection into animals:

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