TABLE II.
Effects of Various Metabolic Agents on Resistance of Rats to Anoxia.

	No. of animals	Avg body wt (g)	Avg survival (min) ± standard error
Controls	20	192	47.3 ± 3.4
Thyroidectomized	20	118	64.0 ± 3.2
Controls	7	279	50.4 ± 2.7
Thiourea	9	155	68.0 ± 3.2
Controls	6	267	44.3 ± 3.2
Thyroxine	6	225	26.5 ± 4.1
Controls	6	193	44.2 ± 3.7
Dinitrophenol	6	197	12.1 ± 4.8

ciency,[15,16] decreases the resistance to low oxygen pressures as does thyroxine. However, in the animals fasted for one day, the effect of metabolic variations on anoxia resistance was overshadowed by the increased sensitivity to anoxia resulting from the fast.[8,9]

Conclusions. (1) Thyroidectomy increases the resistance of the rat to anoxia; but only in fed animals. (2) Treatment with thyroxine decreases the resistance of the rat to anoxia. Since dinitrophenol, a metabolic stimulator without effect on thyroid deficiency, also decreases the resistance to a low oxygen pressure, the role of the thyroid in conditions of anoxia appears mediated through the effect of this gland on metabolism. (3) Animals given a 1% solution of thiourea in drinking water show a marked increase in resistance to anoxia probably as a consequence of the suppression of thyroid function due to this drug.

15 Leblond, C. P., and Hoff, H. E., *Am. J. Physiol.*, in press.

16 Leblond, C. P., in press.

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Inhibition of Growth of Typhus Rickettsiae in the Yolk Sac by Penicillin.

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The results to be reported here were obtained in one of a series of experiments designed to test the effects of various enzyme inhibitors and activators on the intracellular multiplication of rickettsiæ. The yolk sac of the developing hen's egg (Cox[1]) was chosen as the medium for this work because of its uniformity and economy.

Material and Methods. A murine strain of typhus rickettsiæ, in its 8th and 9th passages in fertile eggs, was used. 36 eggs were injected, 18 of which were treated with penicillin, while 18 served as controls. All eggs were incubated at 37°C.

A yellow powder containing the sodium salt of penicillin was dissolved in distilled water. In the first experiment 0.2 cc of this solution, containing 325 Oxford units, was injected into the yolk sacs of 6 eggs on the 2nd, 4th, and 6th days after injection with rickettsiæ. In the second experiment, similar injections were given to 12 eggs on the 3rd, 5th, and 7th days after injection with rickettsiæ.

The eggs were studied for the presence of rickettsiæ at intervals after injection. When an egg in either the control or the treated series showed evidence of fetal death, it was opened for study, and usually an egg in the corresponding series was opened and studied simultaneously. In Experiment 1, when the

1 Cox, H. R., *Science*, 1941, **94**, 399.

TABLE I.
Growth of *Typhus rickettsiæ* in Penicillin-Treated Eggs.
Experiment 1.

Control series				Penicillin series		
Day after inoculation	Egg No.	Degree of infection*	Embryo at time of examination	Egg No.	Degree of infection*	Embryo at time of examination
5				1	(+)?	dead
8	1	++	dead			
9	2	++	alive	2	(+)	alive
10	3	+++	,,			
11	4	++++	dead			
	5	+++++	,,			
12	6	++++	,,	3	(+)	,,
13				4	(+)	,,
16				5	(+)	,,
17				6	(+)	dead

* (+)? No *rickettsiæ* recognizable with certainty.
(+) Less than one *rickettsia* per oil immersion field.
\+ 1-10 *rickettsiæ* per oil immersion field.
++ 10-100 ,, ,, ,, ,, ,,
+++ 100-1000 ,, ,, ,, ,, ,,
++++ 1000-5000 ,, ,, ,, ,, ,,
+++++ 5000-8000 ,, ,, ,, ,, ,,

embryos of the last 3 control eggs died with overwhelming rickettsial infection, the remaining treated eggs were opened subsequently at varying intervals. Under the conditions of the experiment, eggs rarely hatched, even when not given injections of any sort.

The degree of infection was estimated in giemsa-stained smears from the yolk sac membrane of each egg, and occasionally in giemsa-stained paraffin sections. The number of rickettsiæ was determined, in lightly infected eggs, by actually counting the organisms in a number of oil immersion fields. In heavily infected eggs, the rickettsiæ in a small portion of each of several fields were counted, and on this basis an estimate was made of the total number in each field. Although several sources of error are inherent in this method, it gave results sufficiently accurate for the purposes of this study.

On two occasions, material from one control and one treated egg was injected intraperitoneally into guinea pigs, in order to test for virulence. The yolk from one egg showing fetal death 9 days after the last injection of the drug was tested for penicillin activity against *Staphylococcus aureus*.

In order to rule out the possibility that the observed effect might be due to the injected distilled water, a supplementary series of 12 eggs was injected with rickettsiæ, and 6 of these were injected with 0.5 cc of distilled water 5 days later.

Results. From Tables I and II, it will be seen that the majority of the control eggs died with heavy rickettsial infection between the 9th and 13th days after injection. Of the 18 eggs in this series, one died on the 4th day—too early to be significant. In 3 eggs, the estimated number of rickettsiæ per oil immersion field was 10-100; in 2 eggs, 100-1000; in 5 eggs, 1000-5000; and in 7 eggs 5000-8000.

In the penicillin treated series, one fetal death occurred on the 3rd day, one on the 5th day, and 2 on the 6th day; all before the usual period for massive rickettsial growth. (Traumatic injury from successive injections may have caused some of these early fetal deaths.) In 9 eggs, the estimated number of rickettsiæ per field was less than one; in 3 eggs, 1-10; in one egg 100-1000; and in one egg 1000-5000.

Guinea pigs injected with material from 2 control eggs (heavily infected) and 2 treated eggs (containing less than one organism per field) all developed typical murine typhus infection. Penicillin activity could not be demonstrated in the yolk tested 9 days after the last injection of the drug.

Four of the 6 eggs in the supplementary series given injections of pure distilled water all showed massive rickettsial infection, quan-

TABLE II.
Growth of *Typhus rickettsiæ* in Penicillin-Treated Eggs.
Experiment 2.

Control series				Penicillin series		
Day after inoculation	Egg No.	Degree of infection*	Embryo at time of examination	Egg No.	Degree of infection*	Embryo at time of examination
3				1	(+)?	dead
4	1	(+)?	dead			
6				2	(+)?	"
				3	(+)?	"
8	2	+++	alive	4	(+)?	"
9	3	++	"	5	(+)	alive
10	4	++++	dead	6	(+)	"
11	5	++++	alive	7	(+)	"
12	6	+++++	dead	8	+	"
	7	+++++	"	9	++++	"
	8	+++++	"			
	9	+++++	"			
	10	+++++	"			
13	11	+++++	"	10	+++	dead
14	12	++++	alive	11	+	alive
15				12	+	"

* For explanation of symbols, see Table I.

titatively comparable to that seen in the control eggs. The other two died early with bacterial contamination.

Discussion. The positive inoculation test in the case of two eggs in which the number of rickettsiæ in smears was less than one per field, indicates that some of these organisms were alive. It is not clear whether these scattered rickettsiæ indicate a slight multiplication of the organisms, or merely represent the organisms originally introduced.

The eventual development of massive rickettsial infection in 2 of the treated eggs, 5 and 6 days after the last injection of penicillin, is not surprising in view of the evidence, obtained by guinea pig injection, that live organisms were present. The failure of this massive growth to occur with regularity after the cessation of treatment is probably to be explained by the fact that in the later stages of embryonic development, conditions in the entodermal cells lining the yolk sac become less favorable for rickettsial growth. It is probable that the penicillin, as given in these experiments, inhibited rickettsial growth during the critical period, so that the eventual development of massive infection was usually not possible.

It should be emphasized that penicillin, in all probability, is destroyed rather rapidly in the yolk sac, and that no attempt was made to maintain effective concentrations. The drug was given in 3 single doses rather than in many divided doses in order to lessen the danger of traumatic fetal death.

Although these experiments show a striking inhibition of rickettsial growth by penicillin, there is no evidence that rickettsiæ completely disappeared in any instance. The mechanism of the inhibitory effect on rickettsial growth is not clear at present. In general, rickettsial and viral infections are unaffected by chemotherapeutic agents of the sulfonamide group. The only exceptions of which we are aware are lymphogranuloma venereum,[2] mouse pneumonitis,[2] and trachoma.[3] In experimental typhus and spotted fever, negative or even suggestively detrimental effects from the sulfonamides have been reported.[4,5] Preliminary experiments in this laboratory indicate that sulfanilamide does not inhibit rickettsial growth in the yolk sac. Penicillin is reported to be ineffective against the influenza virus, PR8.[6]

Because of the fact that rickettsiæ and

[2] Rake, J., Jones, H. P., and Nigg, C., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 449.

[3] Julianelle, L. A., and Smith, J. E., *Am. J. Ophth.*, 1942, **25**, 317.

[4] Topping, N. H., *Pub. Health Rep.*, 1939, **54**, 1143.

[5] Pinkerton, H., *Bact. Rev.*, 1942, **6** (No. 1), 37.

[6] Robinson, H. J., *J. Pharm. and Exp. Therap.*, 1943, **77**, 70.

viruses are obligate intracellular parasites, studies of the effect of chemotherapeutic agents on these pathogens are complicated by several factors, which need not be considered in the case of ordinary bacteria. Unknown factors are (1) the permeability of living host cells to the agents, (2) their stability within cells, and (3) their effect on the enzyme systems of the cells. Since rickettsiæ and viruses are dependent in variable degrees upon the enzyme systems of the cells in which they grow, any observed effects on the growth of intracellular parasites might be either direct (comparable to the bacteriostatic effect upon free-living bacteria) or indirect, secondary to alterations of the metabolism of the host cells. It cannot be assumed, therefore, that the inhibitory effect of penicillin on rickettsial growth in the yolk sac is brought about necessarily in the way similar to the bacteriostatic effect of this substance on free-living bacteria.

Multiplication of rickettsiæ in the yolk sac takes place only with cells.[7] Penicillin was not injected until 48 or 72 hours after the injection of rickettsiæ, in order to allow time for the injected rickettsiæ to gain entrance into the cells. Careful attention was given to the cells in the yolk sacs of the penicillin-injected eggs, and with the exception of the 2 heavily infected eggs (Table II), only a rare isolated organism was seen in such a position that it might have been thought to be intracellular. The observations made strongly suggest that the inhibition of rickettsial growth is brought about by penetration of the penicillin into the cells, and not entirely by its direct effect on extracellular organisms in the process of passing from cell to cell.

The effect of penicillin injection on experimental typhus and spotted fever infection in mice and guinea pigs is being investigated and will be reported later. The experiments reported here suggest the possibility that penicillin might be effective in these diseases.

Conclusion. Penicillin, injected in 3 doses at intervals of 48 hours, exerted a striking inhibitory action on the multiplication of murine typhus rickettsiæ in the yolk sac of the fertile hen's egg.

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[7] Greiff, D., and Pinkerton, H., to be published.

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Glucose Tolerance of Fasted and Insulinized Chicks.

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The blood sugar level of fasted chicks exhibits a definite upward trend after 24 hours of fast, while 24 hours after large doses of insulin the blood sugar level is elevated above the fasting level.[1] The elevation of the blood sugar level after large doses of insulin is apparent after a single injection and becomes more marked after subsequent injections in either fasted or non-fasted chicks. The insulin sensitivity of such chicks may be tested by administering a small dose of insulin and observing the fall of blood sugar 1½ hours after injection, since the response is a logarithmic function of the dose up to 1.5 units per kilogram. The behavior of the blood sugar curve after large doses of insulin raised the question whether or not such chicks were less sensitive to small amounts of insulin. It has been shown in a previous publication that neither fasting nor dehydration, separately or together, would change the insulin sensitivity.[2] Such a desensitizing effect has been found, and a study of the glucose tolerance on fasted chicks receiving insulin and

[1] Opdyke, D. F., *Endocrinology*, 1942, **31**, 363.

[2] Opdyke, D. F., *Am. J. Physiol.*, 1943, **139**, 563.