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.

For the penicillin used in these experiments, the authors are indebted to the National Research Council Committee on Chemotherapeutics and Other Agents.

[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.

TABLE I.

A comparison of the insulin sensitivity of chicks fasted 72 hours with that of chicks fasted 72 hours and, in addition, receiving 3 large doses of insulin during the fast. The insulin was administered in 20-unit doses at the beginning and after 24 and 48 hours of fast. Insulin sensitivity was determined by injecting 0.18 unit of insulin and measuring the blood sugar 1½ hours afterward.

Experimental condition	No. of chicks	Blood glucose, mg %: Control blood sugar	Blood glucose, mg %: 90 min. after injection of test dose	% change in blood sugar
Fasted 72 hrs. 0.18 u. insulin test dose	29	181 ± 3.5*	140 ± 3.6*	—22.6 ± 1.9*
Fasted 72 hrs. 3 inj. of 20 u. insulin day. 0.18 u. insulin test dose	28	207 ± 10.0	180 ± 12.0	—13.0 ± 3.5

* Mean and standard error.

without insulin was instituted.

Procedure. The chicks used in this study were cockerels of the single comb White Leghorn variety. They were obtained from the hatchery when one day old and were raised on a diet of Wayne or Arcady chick mash to an age of 30-40 days. Their body weight ranged between 200 and 400 g, with an average weight of 260 g.

Blood sugars were determined by the method of Hagedorn and Jensen as given by Peters and Van Slyke.[3] The blood was obtained directly from the wing vein. The chicks were individually marked so that the time between samples could be very accurately controlled.

Glucose (2 g kilo, 10% solution) was administered by introducing the free end of a rubber tube attached to a graduated burette well down into the crop. The amount of glucose solution given was adjusted to the nearest 10 g of body weight. No regurgitation occurred if the tube was carefully withdrawn. A control blood sugar sample was taken immediately before the glucose solution was given. Blood samples were also taken 20, 40, and 80 minutes after the administration of glucose.

Insulin* was injected into the pectoral muscle. The test dose was 0.18 unit in a volume of 0.5 cc of fluid. Dilutions were made with acidified distilled water, pH 2.4.

Results. Tables I and II summarize the results of this study.

Table I clearly indicates that the previous injection of large doses of insulin decreases the sensitivity of chicks to a small dose of the same hormone. The fasted and insulinized chicks exhibit the typical increase of the blood sugar level above the fasting level, and the percentage change in blood sugar following the test dose of insulin is significantly less than that found in the chicks which were only fasted without receiving any insulin previously.

If the data presented in Table II are studied graphically, striking differences in the character of the glucose tolerance curves become obvious. The blood sugar of the non-fasted series has returned to its original level 80 minutes after the ingestion of glucose, the highest level being obtained after 20 minutes. After 24 hours of fast the blood sugar fails to return to its original level at the end of 80 minutes. The difference between the original value and that at 80 minutes is highly significant. In addition, the level of the blood sugar 20 minutes after the administration of glucose is significantly higher in the 24-hour fasted series than in the non-fasted series. The 72-hour fasted group presents a peculiar feature in that the curve is greatly flattened. The blood sugar level fails to reach the level found in the 24-hour fasted series, although it is still above the initial level at the end of the 80-minute period.

The most marked change in the character of the response occurs after administration of large doses of insulin. Here, 24 hours after a single injection of 20 units of insulin, the control blood sugar is elevated; the peak occurs 40 minutes after the administration of

[3] Peters, J. P., and Van Slyke, D. D., 1932, *Quantitative Clinical Chemistry*, Williams and Wilkins.

* The insulin (Iletin) was kindly furnished by Eli Lilly and Co., Indianapolis, Indiana.

TABLE II.

Glucose tolerance of non-fasted, fasted, and insulin-treated fasted chicks. All chicks received 2 g glucose per kilo. The insulinized chicks received one injection of 20 units of insulin per day of fast. The glucose tolerance of the insulin-treated chicks was determined 24 hours after the last insulin injection.

Experimental conditions	No. of chicks	Hours fasted	Blood glucose in mg % after 2 g glucose/kilo: Control before glucose	20 min	40 min	80 min
Non-fasted	10	0	201 ± 3.1*	241 ± 8.1*	211 ± 3.9*	199 ± 4.3*
Fasted only	15	24	179 ± 3.5	274 ± 10.0	220 ± 5.7	193 ± 3.3
" "	15	72	178 ± 2.9	227 ± 7.3	226 ± 8.3	197 ± 4.3
Fasted and receiving 20 units insulin 24 hrs prior to test	5	24	194 ± 27.0	239 ± 14.0	270 ± 22.0	255 ± 34.0
Fasted and receiving inj. of 20 u. insulin at 0, 24, and 48 hrs of fast	15	72	224 ± 13.0	313 ± 14.0	338 ± 13.0	344 ± 6.4

* Mean and standard error.

glucose, and the blood sugar remains markedly elevated at the end of 80 minutes. With 72 hours of fast and three large injections of insulin the shape of the curve is changed even more radically. In the latter case, the blood sugar level is still rising at the end of the 80-minute period. Twenty minutes after the administration of glucose the blood sugar has reached a concentration not attained at any time in the preceding experiments, *i.e.*, 313 mg % as compared with 270 mg %. It is evident that the previous injection of insulin exerts a powerful influence on the character of the glucose tolerance curve.

Discussion. The results of the glucose tolerance tests on fasted chicks suggest the phenomenon of hunger diabetes which is well known in dogs, human beings, and rabbits.[4-6] The theory has been advanced that hunger diabetes results from the failure of the islet tissue of the pancreas to secrete insulin in the absence of ingested carbohydrate. Such a theory is supported by the experiments of Best *et al.*[7] which show that the insulin content of the pancreas of fasted or fat-fed rats is less than that of normally fed rats. A failure of the blood sugar of the fasted chick to return to normal after glucose ingestion might be explained on this basis. However, such an interpretation neither explains the marked flattening of the glucose tolerance curve after 72 hours fast, nor does it explain why the level of the blood sugar fails to reach the height attained after comparable treatment at the end of a 24-hour fast.

The very marked decrease in tolerance to glucose exhibited by chicks previously injected with large doses of insulin indicates a major change in the operating economy of these animals. Similar results have been reported on rabbits when glucose was given immediately after recovery from a hypoglycemia induced after injection of 1½-2 units of insulin.[6] Conceivably, such experimental conditions may upset the dynamic balance between the production of carbohydrate from endogenous sources and the utilization of that carbohydrate. Whether fasting decreases the utilization of carbohydrate is still a debatable question. The experiments of Bergman and Drury[8] on eviscerated fasted and fed rabbits indicates a lowered utilization rate of carbohydrate in the fasted animals. The progressively rising blood sugar level of the fasted chick and the augmentation of this rise by a single or repeated injection of insulin may be interpreted as an increase in the production

[4] Dann, M., and Chambers, W. H., *J. Biol. Chem.*, 1930, **89**, 675.

[5] Goldblatt, M. W., and Ellis, R. W. B., *Biochem. J.*, 1932, **26**, 991.

[6] du Vigneaud, V., and Karr, W. G., *J. Biol. Chem.*, 1925, **66**, 281.

[7] Best, C. H., Haist, R. E., and Ridout, J. H., *J. Physiol.*, 1939, **97**, 107.

[8] Bergman, H. C., and Drury, D. R., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 414.

of carbohydrate, a decrease in its utilization, or both. Possibly, both effects are present at different times, the overproduction phase representing a secondary reaction to the injection of insulin and manifesting itself as an increased blood sugar 24 hours after the last injection of insulin.

Summary. Normal chicks exhibit the usual type of glucose tolerance curve, but one shorter in duration when compared with the mammalian curves. The character of the curve begins to change after 24 hours of fast and is markedly changed when taken after 72 hours of fast. Previous injections of large doses of insulin greatly decreases the glucose tolerance.

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Influence of Atmospheric Temperature upon Reaction of Rabbits to Insulin.*

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The higher mortality among white rats and white mice when kept at atmospheric temperatures considerably higher than normal, after receiving injections of insulin, and their increased inclination to convulse has frequently been reported.[1-5] Others have found, however, that the inclination of white mice to convulse is the same at 30°C as at 18°-20°C.[6] It has also been found[7] that rabbits acclimated at a temperature of 30°C required but one-half as much insulin to produce the same percentage of blood sugar reduction as those at 22°C.

It was observed in the present experiments that rabbits which responded with mild convulsions, when injected with a prescribed amount of insulin at normal room temperatures, collapsed completely with the first onset of convulsions and expired forthwith after being injected with the same amount of insulin during extremely hot weather (31° to 34°), if not given immediate intravenous injections of glucose. The rabbits used were divided into 3 groups, A, B, and C. Those of group C were inclined to convulse after injections of 0.375 units of insulin per kg of body weight, those of group B with 0.5 units, while those of group A did not convulse with the larger amount. They were used but once a week and were fasted for 18 hours before each experiment. The dosage of insulin in each of these experiments was 0.375 units of crystalline zinc insulin† per kg of body weight.

Samples of blood were taken from each animal just before it was injected with insulin and similar samples taken each hour thereafter for 7 hours to determine the nature of the subsequent changes in the blood sugar level. Blood sugar determinations were made on individual samples according to the method of Miller and Van Slyke.[8] Group averages of the data thus obtained are represented by the graphs of Fig. 1.

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2 Voegtlin, C., Dunn, E. R., and Thompson, J. W., *U. S. Public Health Rep.*, 1924, **39**, 1935.

3 Hemmingsen, A. M., and Krogh, A., *Publications of the Health Organizations of the League of Nations*, C. H. 398, 40.

4 Trevan, J. W., and Boock, E., *Publications of the Health Organizations of the League of Nations*, C. H. 398, 46.

5 Hemmingsen, A. M., *Skand. Arch. Physiol.*, 1939, **82**, 105.

6 Horsters, H., and Brugsch, H., *f. d. ges. exp. Med.*, 1929, **65**, 569.

7 Private communication, Lilly Research Laboratories.

† The generosity of the Eli Lilly Company who donated this insulin is gratefully acknowledged.

8 Miller, B. F., and Van Slyke, D. D., *J. Biol. Chem.*, 1936, **114**, 583.