4 hours. At later intervals from 8 hours to 10 days after the reinfection, viable parasites could no longer be recovered by culture from the majority of animals.

1. Schnitzer, R. J., Kelly, D. R., and Leiwant, B., *J. Parasitol.*, 1950, v36, 343.

2. Kelly, D. R., and Schnitzer, R. J., *J. Immunol.*, 1952, v69, 337.

3. Johnson, G., and Trussell, R. E., PROC. SOC. EXP. BIOL. AND MED., 1943, v54, 245.

4. Culbertson, J. T., *Immunity against animal parasites*, Columbia University Press, N. Y., 1948.

5. Nelson, P., Trichomonas foetus: Infection, immunity and chemotherapy, unpublished Thesis Univ. Wisconsin, 1938.

Received February 2, 1953. P.S.E.B.M., 1953, v82.

## Alpha Cell Damage and Blood Sugar Changes in Rabbits after Administration of Cobalt.* (20130)

BRUNO W. VOLK, SYDNEY S. LAZARUS, AND MARTIN G. GOLDNER.

*From the Division of Laboratories and the Department of Medicine, Jewish Sanitarium and Hospital for Chronic Diseases, Brooklyn, N. Y.*

In a preliminary report it has been shown by us that in the rabbit a single intravenous injection of cobaltous chloride causes both transitory hyperglycemia and severe selective damage to the pancreatic alpha cells(1). Although the blood sugar returned to the pretreatment level within a few hours after cobalt administration, the alpha cell damage was still present after 48 hours. Despite these persistent anatomical changes, a second dose of cobaltous chloride administered at 48 hours induced a second hyperglycemic episode. These observations suggest two possible mechanisms for the action of cobalt. On the one hand it might be assumed that the alpha cells on being damaged by cobaltous chloride released a factor with hyperglycemic properties. This hypothesis would be in conformity with numerous reports which give evidence for the presence of a hyperglycemic glycogenolytic factor (HGF) in the pancreas, and which suggest that the alpha cells are the site of its production(2-4). The absence of a persistent hypoglycemia after the initial hyperglycemia and the repeated hyperglycemic episodes elicited by additional injections of cobaltous chloride may indicate simply that alpha cells sufficient to maintain blood sugar homeostasis and to cause a hyperglycemic phase on readministration of cobaltous chloride have escaped the damaging effects of the initial injection. On the other hand, the alternate hypothesis has to be considered that the alpha cell damage and the hyperglycemic episodes are independent effects of cobaltous chloride, the latter being of extrapancreatic origin. The experiments to be reported in this paper were undertaken to study the interrelationship between the anatomical and functional changes caused by cobaltous chloride.

*Material and methods.* Cobaltous chloride as 0.5% solution in saline was administered intravenously to normal and alloxan diabetic rabbits of both sexes weighing from 2000 to 3000 g. Alloxan diabetes was produced by the intravenous injection of 100 mg/kilo of alloxan monohydrate (Eastman). Those animals which maintained blood sugar levels over 300 mg for at least one week were utilized. Blood samples for glucose determination by the Nelson modification of the Folin-Wu Micro Method(5) were withdrawn from the marginal ear vein. Animals were sacrificed by intracardiac air embolization and portions of the pancreas and liver were removed for histological examination, which included formalin fixed paraffin sections of both organs stained with Haematoxylin and Eosin, Bouin fixed pancreas prepared by Gomori's Method (6), and alcohol fixed liver stained by the MacManus technique(7).

* This study was aided in part by a grant from the Ciba Pharmaceutical Products Inc., Summit, N. J.

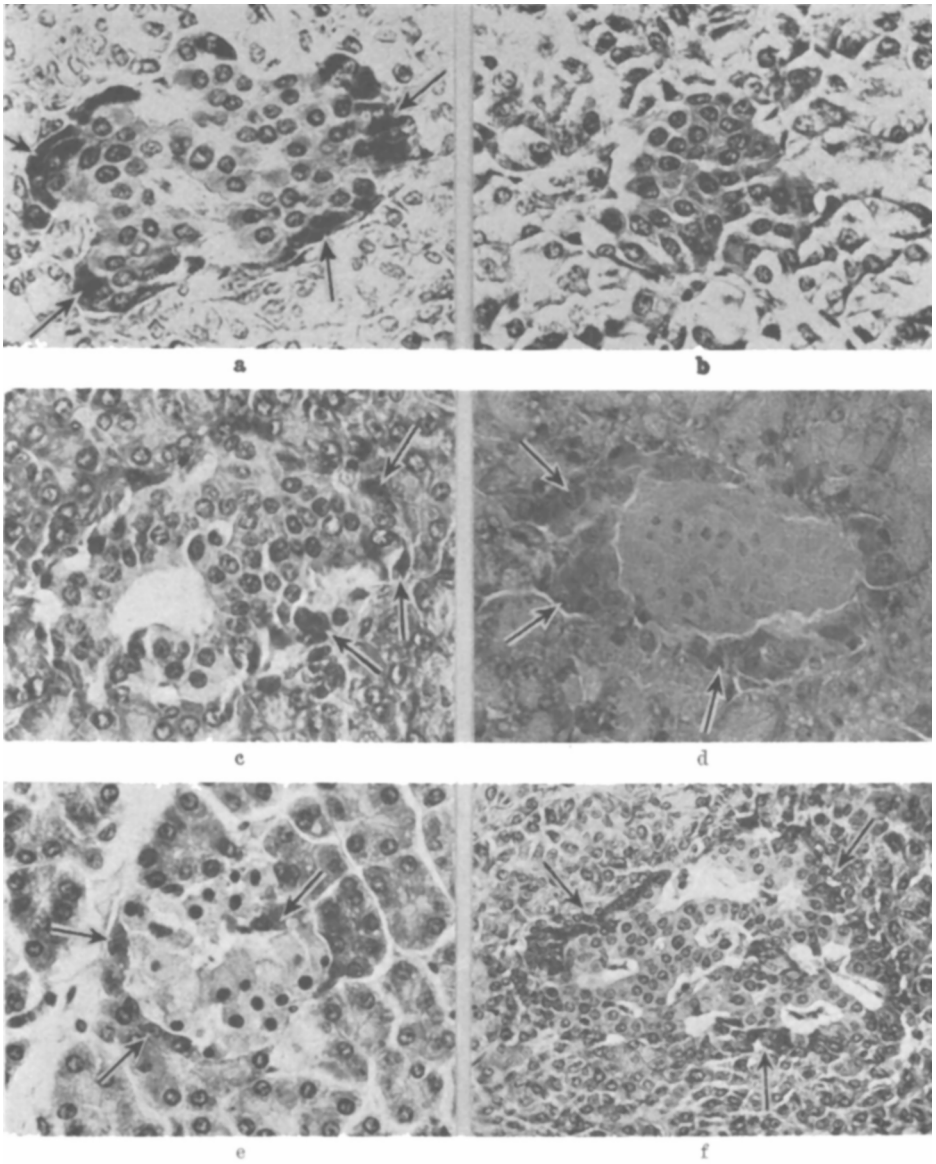

FIG. 1. a. Pancreatic islet of rabbit 1 hr after 50 mg of $CoCl_2$. Arrows indicate degenerating alpha cells. Gomori Stain × 500. b. Same, 3 hr after 50 mg of $CoCl_2$. The islet is composed exclusively of beta cells. Gomori Stain × 500. c. Sections from same pancreas as b, showing a few degenerated alpha cells (arrows). Gomori Stain × 500. d. Pancreatic islet from alloxanized rabbit. Arrows indicate intact alpha cells surrounding debris of the beta cells. Gomori Stain × 500. e. Pancreatic islet from alloxanized cobalt-treated rabbit. Arrows indicate remnants of alpha cells. Gomori Stain × 500. f. Normal control. Gomori Stain × 375.

*Results.* a. *Optimal Dosage and Toxicity.* An initial series of normal animals each received single intravenous injections of either 20, 40, 50, 80, 100, or 200 mg of cobaltous chloride. The blood sugar fluctuations and morphologic changes were followed as long as the survival of the animals permitted. Those animals receiving 100 mg or more developed wheezing, diarrhea and convulsions and usually died within several hours with symptoms of severe respiratory distress. Animals receiving less than 100 mg of cobalt occasionally

showed diarrhea but no other toxic symptoms. The hyperglycemic response and histologic changes after 20 mg of cobalt were inconstant. Doses of 40 mg to 50 mg seemed to give optimal results. Therefore, a dose of 50 mg was used routinely for the rest of the experiments.

b. *Effect of single and repeated injections of 50 mg of cobaltous chloride.* A single injection of 50 mg of cobalt was given to normal animals and blood samples were withdrawn at half hour intervals for 4 hours, and daily thereafter. Two animals each were sacrificed at 1, 2, and 3 hours, and 1, 2, 4, 6, and 10 days after cobalt administration. Additional groups each received repeated injections of 50 mg of cobalt: 1) at one hourly intervals for 4 doses; 2) at 2 hourly intervals for 3 doses; and 3) at daily intervals for 3, 4, 7, or 10 doses. Animals receiving 3 or more injections of 50 mg each at 1- or 2-hour intervals usually did not survive the third injection. In each case in surviving animals blood sugar determinations were done every half hour during the period immediately following the cobalt injections and at daily intervals thereafter.

*Morphologically* it was found that one hour after a single injection of 50 mg of cobaltous chloride there was considerable diminution in the number of alpha cells. Most of those remaining were markedly shrunken and rounded in appearance (Fig. 1a). The granules had disappeared and the cytoplasm had become homogeneously acidophilic. The nuclei were compact and pyknotic. Two or three hours after the injection there was a further decrease in the number of alpha cells and many of those still visible were transformed into clumps of acidophilic debris. Many islets at this time consisted only of beta cells (Fig. 1b). In other islets occasional isolated "shadows" of alpha cells were seen at the periphery (Fig. 1c). However, a few intact alpha cells were still visible in most sections. This picture became increasingly accentuated until 48 hours after the cobalt administration. After 4 days these changes were still present. Although after the 6th day the cellular damage was still marked, the number of intact alpha cells was increased, and by the 10th day the regenerative processes were more marked. It should be noted that the effect of cobalt is not entirely uniform in different animals. Since repeated injections of cobalt at hourly and 2-hourly intervals usually caused the death of the animal within 6 hours, serial histologic studies could not be done. However, the most marked alpha cell damage was observed in pancreatic tissue obtained from these animals. Daily administration of cobaltous chloride, on the other hand, did not seem to intensify the anatomical changes produced by a single injection.

Histological examination of the livers failed to reveal any parenchymatous damage but showed marked depletion of glycogen in almost every instance.

The *blood sugar level* after a single intravenous dose of 50 mg of cobalt reached its maximum value between 30 minutes and 2 hours after the injection. The peak blood sugar value averaged 60 mg % above the fasting level. Within 4 hours the blood glucose concentration reverted to the normal range (Fig. 2a). Subsequent daily blood sugar values were not significantly different from those

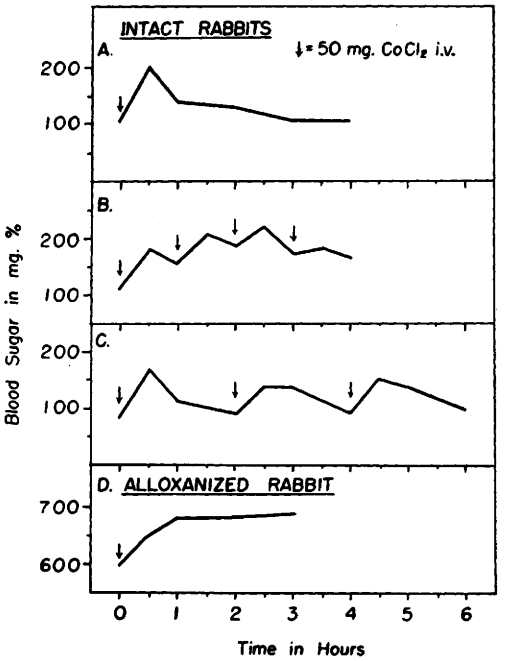


FIG. 2. Initial effect on blood sugar level of single or multiple injections of cobaltous chloride in fasting normal and alloxanized rabbits.

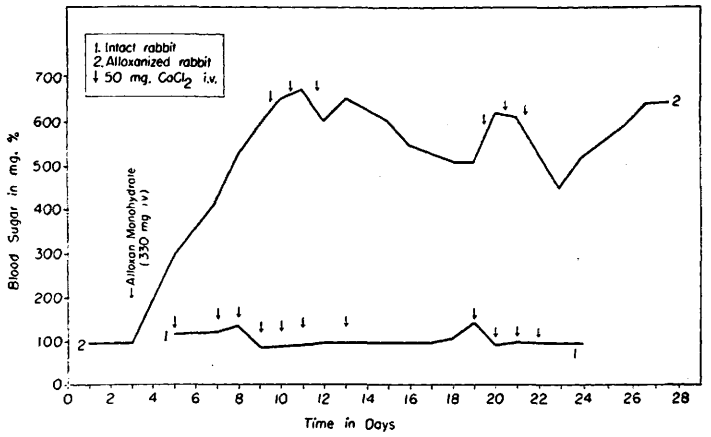


FIG. 3. Effect of daily injections of cobaltous chloride on fasting blood sugar level of normal and alloxanized rabbits.

which would be found in untreated normal animals. Fig. 2b demonstrates the effect on the blood sugar level of consecutive hourly administrations of cobalt, while Fig. 2c represents the effect of injections at two hourly intervals. These repeated injections each cause a rise of the blood sugar level. Repeating the injection of cobalt at daily intervals caused each time a transitory rise of the blood sugar level similar to that seen in Fig. 2a. The interval blood sugar, as seen in Fig. 3, Curve 1, did not vary significantly from the physiologic range.

c. *Effect of cobalt on the alloxanized rabbit.* The alloxan diabetic animals received either single injections of 50 mg cobaltous chloride or repeated injections at daily intervals for 3 days. No insulin was given prior to or during the experiment. Blood samples were taken every half hour for a four-hour period after each cobalt injection and daily thereafter. Animals were sacrificed serially. In several animals the same experiment was repeated at 10-day to 2-week intervals. The islets of control alloxanized rabbits showed the usual morphologic picture consisting of a center composed of granular debris of degenerating beta cells with a peripheral layer of intact alpha cells (Fig. 1d). After cobalt administration this rim of alpha cells showed the same morphologic changes as occurs in the normal islet (Fig. 1e). Most of the alpha cells were replaced by amorphous cytoplasmic remnants. However, it seemed that this destruction of the alpha cells was more marked in the alloxan diabetic than in the normal rabbit. One of our slides showed only undifferentiated cellular debris with an occasional cluster of degenerated alpha cells in the islets.

The effect of cobalt chloride on the blood sugar level of the diabetic animal showed two different phenomena: firstly, each injection was followed immediately by an augmentation of the hyperglycemia which was more prolonged but of the same degree as in the normal rabbit (Fig. 2d); secondly, after the second or third cobalt injection there occurred in some animals a gradual fall of the fasting blood sugar. This continued for several days. After four or five days the blood sugar level, however, returned gradually to the pre-cobalt level (Fig. 3, Curve 2). The alloxanized animal seemed to be more sensitive to the toxic action of cobalt than the normal rabbit and occasionally died after the second or third daily injection with a precipitous fall in the blood sugar.

*Discussion.* The data presented in this paper favor the hypothesis that the transitory hyperglycemia and the selective damage to the pancreatic alpha cells are independent effects of the intravenous administration of cobaltous chloride.

The morphologic studies demonstrate that the alpha cell damage appears rapidly and is rather extensive. It is observed within one hour after cobalt administration and persists for a period of at least 10 days. Only a few

cells escape serious morphologic damage. Regenerative processes seem to start by the sixth day. The damage induced by a single intravenous dose of 50 mg appears to be almost optimal and can be increased only if the injections are repeated at short intervals of one or 2 hours. Injections at intervals of one or more days do not seem to augment the damage elicited by the first injection.

The changes of the blood sugar, on the other hand, revealed an entirely different pattern. Each intravenous injection was followed by a transitory hyperglycemia, regardless whether the time interval was one or two hours, or 1, 2, or 10 days. The glycemic curve was of the same character and degree after the first injection which caused the most extensive cellular damage as after the subsequent injections which increased this damage only slightly or not at all. Moreover, in the normal rabbit transitory hyperglycemia was followed by a return to normoglycemia within two to four hours in spite of persistent impairment of the alpha cells. This normoglycemia was then maintained indefinitely, or until a new injection caused a new transitory elevation. No periods of hypoglycemia were observed, except in terminal cobalt alloxan diabetic animals, as mentioned above.

If the transitory hyperglycemia were a function of the alpha cell damage, a relationship between its degree and the extent of the cellular damage would be expected; repeated injections of cobalt should cause smaller or no elevations of the blood sugar. The reestablishment and maintenance of normoglycemia in spite of persistent alpha cell damage seems to indicate also that blood sugar homeostasis does not depend on the integrity of these cells unless it is assumed that the few remaining cells suffice for this function.

In the alloxanized animals we found a temporary improvement after cobalt administration, but within a few days the hyperglycemia had returned to the pre-treatment level. It seems possible that a toxic effect on the liver rather than the alpha cell damage caused this temporary lowering of the blood sugar. This is even more likely in view of the death of some of the cobalt treated alloxanized animals with toxic symptoms and a precipitous fall in the blood sugar level. It is well known that hepatic injury ameliorates the diabetes of depancreatized animals(8); reference should also be made to the fact that cobalt is excreted by the organism through the liver and bile(9). It has been stated above that the livers of cobalt treated rabbits appear to be markedly depleted of glycogen.

Our observations are at variance with those of others who noted permanent improvement of alloxan diabetes after the administration of cobalt(10), and who consider the cobalt hyperglycemia as evidence of an alpha cell hormone (11). Further studies are in progress in our laboratory to elucidate the mechanism of the cobalt hyperglycemia in relation to the function of the pancreatic alpha cells as well as on the process of regeneration of these cells after cobalt damage.

*Summary.* 1. The intravenous injection of cobalt chloride is accompanied by a transient hyperglycemic episode and causes rapid selective injury to the alpha cells of the pancreatic islets which can still be observed after 10 days. However, regeneration of the alpha cells starts by the 6th day. 2. Since repeated cobalt injections each elicit a similar change in the blood sugar level, this is thought not to be due to the alpha cell damage but to an extrapancreatic toxic action of cobalt probably on the liver. 3. Hypoglycemia is not observed in normal rabbits with alpha cell damage, suggesting that the blood sugar homeostasis does not depend on the integrity of these cells. 4. Although a lowering of the blood sugar which is apparently a toxic effect, is observed in alloxan diabetic animals for a few days after repeated cobalt injection, the pre-injection level is regained while alpha cell damage is still present. This is interpreted to signify that the severity of the diabetes of the alloxanized animal is not a result of a physiologic hyperglycemic action of the alpha cells.

The authors wish to acknowledge the technical assistance of Mrs. H. F. Crowley. The photomicrographs were taken by Mr. H. A. Fischler.

1. Goldner, M. G., Volk, B. W., and Lazarus, S. S., *Metabolism,* 1952, v1, 544.

2. Buerger, M., and Brandt, W., *Z. f. d. Ges. Exp. Med.,* 1935, v96, 375.

3. Pincus, I. J., *J. Clin. Endocrin.*, 1950, v10, 556.
4. Sutherland, E. W., and de Duve, C., *J. Biol. Chem.*, 1948, v175, 663.
5. Nelson, N., *J. Biol. Chem.*, 1944, v153, 375.
6. Gomori, G., *Am. J. Path.*, 1941, v17, 395.
7. McManus, J. F. A., *Nature*, 1946, v158, 202.
8. Soskin, S., and Levine R., *Carbohydrate Metabolism*, Univ. of Chicago Press, Chicago, Rev. Ed., 1952, pg. 209.
9. Greenberg, D., and Copp, H. D., *J.B.C.*, 1943, v147, 749.
10. Van Campenhout, E., and Cornelis, G., *C. R. Soc. Biol.*, Paris, 1951, v145, 933.
11. Avezzu, G. e Luise, R., *Boll. Soc. Ital. Biol. Sper.*, 1951, v27, 1515.

Received February 2, 1953. P.S.E.B.M., 1953, v82.

## Organelle Nature of a Cell Granule from a Thermophilic Bacterium.* (20131)

LOUISE BURNS AND WALTER MILITZER. (Introduced by C. E. Georgi.)
*From the Department of Chemistry, University of Nebraska, Lincoln, Neb.*

Recent interest in mitochondria of animal and plant cells has quickened the search for specific granules in bacteria(1). In a former paper from this laboratory(2), we presented evidence to show that a fraction isolated from a thermophilic bacterium(3), *Bacillus stearothermophilus* (No. 2184), was, indeed, a specific cell granule. The data rested upon photomicrographs and upon certain enzyme data. Some doubt could still be raised at the organelle nature of the granules on the basis that the particles isolated are not the particles as seen in the intact cells. The percentage of granules in whole cells is roughly 60% of the total protein, a figure which is entirely out of proportion by comparison to figures for organelles of animal cells. Further, staining technics on whole cells could lead to false interpretations due to diffusion artifacts, a fact well recognized by Koelle(4) and Novikoff(5).

Recently, we have obtained data by the use of triphenyltetrazolium chloride (TPTZ) that leaves little doubt as to the existence in the intact cells of the granules obtained after treatment with lysozyme.

Cells of the thermophilic bacterium No. 2184 when treated with TPTZ and substrate reveal zones in the areas where oxidation is carried on due to the deposition of insoluble formazan which can be seen with the phase microscope as highly refractile and remarkably clear areas. Bielig, Kausche, and Haardick(6) have already pointed out the usefulness of TPTZ with the conventional microscope in locating areas of oxidation in bacteria. The granules isolated after lysozyme treatment show the same properties toward TPTZ as do the original cells. In each case where formazan is seen within the cell, the same deposition can be seen in the granule. When no formazan is deposited in the cell, no formazan is deposited in the granules.

The most striking illustration of such parallel behavior is seen with the pyruvic oxidase system. This system oxidizes pyruvate in a solution containing fresh cells or fresh granules (10 mg), diphosphothiamine (50 $\mu$g), pyruvate (0.05 M), TPTZ (0.05%), phosphate buffer of pH 6.5 (1.0 ml), magnesium sulfate ($4 \times 10^{-2}$ M) in a total volume of 3.0 ml. At 60°C the reaction time to show pronounced color development is rapid with granules (15 min.) and somewhat slower with whole cells (30 min.). When viewed under the phase microscope after the reaction, the cells show definite refractile areas (Fig. 1). These areas appear to be in the granules themselves, although distinct discernment of the granule is not quite possible without counterstaining, a practice that destroys the for-

* Aided by a grant from the Division of Research Grants, U. S. Public Health Service. Phase contrast accessories were made available through a grant from the Louis Livingston Seaman Fund, New York Academy of Medicine.