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# THE METABOLISM OF RADIOACTIVE COBALT ($Co^{60}$) IN NORMAL AND ALLOXAN DIABETIC RATS

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# THE METABOLISM OF RADIOACTIVE COBALT ($Co^{60}$) IN NORMAL AND ALLOXAN DIABETIC RATS.*

Frank Ulrich and D. Harold Copp

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Although there are only trace amounts of cobalt in the body, it is known that cobalt, in both plants and animals, is essential to the life of the organism. The role which this metal plays in the metabolism of the body is not known. However, the advent of radioactive tracers and their application to the study of mineral metabolism has brought forth new facts which may eventually explain the processes by which cobalt is metabolized in the body.

Copp and Greenberg (1) using radioactive cobalt showed that the chief path of cobalt excretion in rats is the urine, and that after the administration of even minute amounts, most of the cobalt was rapidly eliminated from the body. It was found by Greenberg, et. al. (2) that bile also serves as an important route for the excretion of cobalt from the body. Sheline (3) confimed this observation using dogs with bile and pancreatic fistulas. He showed that after an intravenous injection of radioactive cobalt, appreciable amounts of the cobalt appeared in bile but not in pancreatic juice.

* This work was supported in part by the Atomic Energy Commission.

Bertrand (4) demonstrated that the concentrations of cobalt and nickel in insulin was often hundreds of times greater than in the tissues from which it was extracted. Furthermore, after Abel and his colleagues (5) succeeded in isolating crystalline insulin, Scott (6) showed that this crystalline material was a salt of insulin in combination with a divalent metal such as cobalt, cadmium, nickel, or zinc. In a series of studies on rats and cattle, Comar and his co-workers (7,8) observed that the liver and pancreas took up relatively large amounts of the injected cobalt in comparison to the other organs of the body. This suggested the possibility that the cobalt in the pancreas might be related to the insulin content of that organ.

The following series of experiments were carried out to study the distribution of cobalt in the rat during the first 24 hours after intraperitoneal injection. A further object was to determine whether alloxan diabetes would have any effect on the uptake of radioactive cobalt by the pancreas.

## METHOD

Rats with alloxan diabetes and normal controls were used for these experiments. The diabetic condition was produced by fasting mature female rats of the Long-Evans strain for 24 hours and then injecting them intraperitoneally with a 5 percent aqueous solution of alloxan. The dose administered was 150 milligrams per kilogram of body weight. If within the first two days an alloxan injected rat did not show at least a 1 plus Benedict reaction of the urine, it was again fasted for 24 hours and given a second intraperitoneal injection of 150 milligrams of alloxan per kilogram of body weight. If the urine after this second dose did not show a 1 plus Benedict reaction, the animal was discarded.

Two weeks after the initial alloxan injections had been made, five alloxan diabetic rats and five normal controls were anesthetized with ether and injected intraperitoneally with 52 micrograms of cobalt tagged

with $Co^{60}$. The cobalt was in the form of $CoCl_2$ dissolved in 1 c.c. of a 0.9 percent solution of saline and contained approximately 1 microcurie of $Co^{60}$ per dose.

After injection each rat was placed in a large glass beaker which was provided with a wire screen for separation of the urine and feces. At specified time intervals - 0.5, 1, 2, 8, and 24 hours - after injection each rat was sacrificed with chloroform. Blood was obtained by heart puncture from the opened thoracic cavity. The spleen, pancreas, liver, kidneys, carcass, and feces were taken for analysis of radioactive cobalt. The inside of the beakers and the wire screens were thoroughly rinsed with 2N HCl and the resulting washings were added to the previously collected urine. After the bladder had been ligated and removed, it was washed out with 2N HCl, the contents being added to the rest of the urine.

With the exception of the blood, the other tissues and excreta were ashed, dissolved in hot 2N HCl, and made up to volume with distilled water. Suitable aliquots were placed in one-half ounce gold-lacquered tin ointment capsules, dried, and the radioactive cobalt present was determined with a Geiger-Müller counter.

Suitable aliquots of whole blood were placed in tin ointment capsules and treated in a similar manner. The total amount of radioactive cobalt in the blood was calculated from the value for blood volume of 4.59 c.c. per 100 grams of body weight given by Berlin (9). This figure was obtained for normal rats using both $Fe^{59}$ labelled red blood cells and $P^{32}$ labelled cells.

In order to study visually the distribution of $Co^{60}$ in the pancreas of the rat the following experiment was performed.

Pieces of pancreas stained supravitally with neutral red were removed from normal and alloxan diabetic rats 1/2 and 1 hour after they had received

an intraperitoneal injection of 52 micrograms of labelled cobalt. A modification of the Haist technique (10) was used for visualizing the islets of Langerhans. Small pieces of the pancreas were squashed between a slide and cover glass, Agfa No-Screen X-ray film was affixed to the preparation and the film was developed 25 days later.

RESULTS

Table I is a summary of the data obtained. The figures represent the average value from 4 or 5 animals expressed as the percent of the injected $Co^{60}$.

The data shows that of the five tissues and organs studied, the liver, in absolute terms, took up the largest amount of cobalt. Next in order of magnitude is the pancreas, although at 8 and 24 hours the amount remaining in this organ is almost the same as that in the kidneys and blood. Both of these tissues have a very similar cobalt content throughout the 24 hour period following injection and are intermediate in magnitude between the pancreas and spleen.

The excretion of coblat by the kidneys is very rapid, almost 70 percent of the injected dose being recovered in the urine at the end of one day. In contrast to this, not more than 2.5 percent of the injected dose is found in the feces at the end of 24 hours.

There does not seem to be any significant statistical difference between the cobalt content of the pancreas in the alloxan diabetic animals and the norma l controls. Neither do the liver, kidneys, spleen, and blood show any significant difference in the cobalt uptake between the two groups. This is also true of the urine and feces.

Figure 1 gives a graphic representation of the distribution of labelled cobalt in the liver, pancreas, blood, kidneys, spleen, urine, and carcass

TABLE I

Distribution of $Co^{60}$ in Various Organs and Excreta of Normal and Alloxan Diabetic Rats

| Time of sacrifice after injection | 1/2 hr. | | 1 hr. | | 2 hrs. | | 8 hrs. | | 24 hrs. | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Alloxan | Control | Alloxan | Control | Alloxan | Control | Alloxan | Control | Alloxan | Control |
| Liver | 31.2±2.8* | 27.6±4.5 | 28.3±1.8 | 34.7±3.8 | 19.8±1.7* | 21.2±3.3* | 11.0±1.1* | 9.3±0.5 | 5.0±0.9 | 6.3±0.8 |
| Pancreas | 8.5±1.9* | 9.3±0.7 | 7.4±0.2 | 8.9±1.5 | 3.6±0.3* | 3.7±0.4* | 1.6±0.1* | 1.6±0.1 | 0.5±0.0 | 0.5±0.0 |
| Spleen | 0.4±0.0* | 0.6±0.0 | 0.4±0.0 | 0.3±0.0 | 1.7±0.4* | 1.9±0.3* | 0.1±0.0* | 0.1±0.0 | 0.1±0.0 | 0.1±0.0 |
| Kidneys | 3.2±0.3* | 3.0±0.3 | 3.3±0.6 | 4.2 ±0.3 | 3.2±0.7* | 1.7±0.1* | 1.1±0.0* | 1.1±0.0 | 1.3±0.5 | 0.8±0.2 |
| Blood | 3.0±0.3* | 2.4±0.3 | 1.8±0.3 | 1.7±0.2 | 1.5±0.1* | 1.6±0.3* | 0.9±0.2* | 1.5±0.2 | 0.5±0.0 | 0.6±0.0 |
| Urine | 6.8±2.8* | 8.5±1.3 | 22.3±2.4 | 19.5±1.4 | 28.3±6.6* | 37.6±5.7* | 52.9±1.2* | 64.3±3.1 | 68.0±5.2 | 65.4±2.1 |
| Feces | 0.0±0.0* | 0.1±0.0 | 0.0±0.0 | 0.2±0.1 | 1.2±0.4* | 0.5±0.2* | 0.6±0.4* | 0.8±0.3 | 1.3±0.4 | 2.5±1.0 |
| Carcass | 45.5±4.7* | 47.2±3.6 | 33.3±3.1 | 31.6±3.1 | 22.8±2.8* | 18.3±2.5* | 13.5±2.1* | 11.4±0.6 | 8.0±1.1 | 8.3±0.7 |
| Total Recovery | 98.6±2.2* | 98.7±1.4 | 96.8±2.7 | 101.1±2.5 | 82.1±3.7* | 86.5±3.4* | 81.7±1.0* | 90.1±3.1 | 84.7±3.4 | 84.5±1.8 |

Values given are expressed as percent of the injected $Co^{60}$ for 5 animals ± the standard error.

*Average value for 4 animals.

at 1/2, 1, 2, 8, and 24 hours after injection, each point on the graph being the average value from 4 or 5 animals.

From this graph it can be seen that the general shape of the curves for the distribution of $Co^{60}$ in the liver, pancreas, kidneys, and spleen is very similar. There is a relatively rapid decrease in the cobalt content of those organs within the first two hours after injection. This decrease gradually becomes less rapid from 2 to 24 hours, the curves for the kidneys and spleen leveling off at around 8 hours.

Although the curve for blood resembles that of the other organs, there is a tendency for the curve to fall extremely rapidly during the first hour. Only the pancreas exhibits a similar rapid fall within the same time interval.

The curve for the carcass is inversely related to the curve for urinary excretion. In the latter there is a rapid rise within the first two hours which then levels off.

Table II is a summary of the specific tissue activity expressed as percent of dose of radioactive cobalt per gram of tissue. Values are given for the liver, pancreas, kidneys, spleen, blood, and carcass at the five time intervals studied in the experiment. A graphic representation of this data is given in Figure 2.

The pancreas has the highest specific activity, with the liver, kidneys, spleen, blood, and carcass having values decreasing in that order. The values for the kidneys, liver, and pancreas although quite different up to 8 hours, begin to converge from that time on and are practically identical at 24 hours.

The radioautographs of the pancreas failed to show any localization of the $Co^{60}$ within the islets. Wherever a given portion of the slide contained some pancreatic tissue from either a diabetic animal or a normal control, the corresponding section of the autograph showed a diffuse blackening with no indication of any selective localization. From this result together with the

TABLE II

Specific $Co^{60}$ Tissue Activity of Various Tissues and Organs of Normal and Alloxan Diabetic Rats

| Time of sacrifice after injection | 1/2 hr. | | 1 hr. | | 2 hrs. | | 8 hrs. | | 24 hrs. | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Alloxan | Control | Alloxan | Control | Alloxan | Control | Alloxan | Control | Alloxan | Cont. |
| Liver | 2.4* | 2.0 | 2.3 | 2.6 | 1.6* | 1.8* | 0.9* | 0.7 | 0.4 | 0.5 |
| Pancreas | 7.0* | 7.1 | 6.1 | 6.8 | 3.0* | 3.3* | 1.4* | 1.3 | 0.4 | 0.4 |
| Spleen | 0.6* | 0.8 | 0.6 | 0.4 | 2.8* | 3.1* | 0.1* | 0.1 | 0.2 | 0.1 |
| Kidneys | 1.4* | 1.2 | 1.6 | 1.9 | 1.5* | 0.8* | 0.5* | 0.5 | 0.6 | 0.3 |
| Blood | 0.15* | 0.08 | 0.08 | 0.08 | 0.08* | 0.08* | 0.05* | 0.08 | 0.02 | 0.03 |
| Carcass | 0.19* | 0.19 | 0.15 | 0.13 | 0.10* | 0.09* | 0.06* | 0.05 | 0.04 | 0.04 |

Unless otherwise indicated, each figure represents the average value for 5 animals.

*Average value for 4 animals.

results obtained from the general distribution studies, it appears that the cobalt content of the pancreas is not concentrated in any particular region of that organ, nor is it affected by the action of alloxan.

## DISCUSSION

The rapid excretion of cobalt in the urine within the first 24 hours confirms the observations of Copp and Greenberg (1).

All of the organs investigated showed a relatively rapid uptake of the isotope and an equally rapid loss from these tissues, suggesting a high turnover rate at this dose level. No quantitative estimations of these rates were made because the number of experimental points on the graph were too few to permit accurate analysis of the curves.

The pancreas, liver, and kidneys showed a relatively high specific tissue activity which confirms the findings of Comar and his co-workers (7,8) that the liver and pancreas took up relatively large amounts of the injected cobalt in comparison to the other organs of the body.

From the data it would appear that alloxan diabetes had no significant effect on the distribution and excretion of radioactive cobalt in these animals. Furthermore, the results of the general distribution study of cobalt in the pancreas and the radioautographs of that organ fail to provide any evidence suggesting that this metal might be concentrated in the islets of Langerhans. It appears probable, therefore, that the cobalt is distributed throughout the acinar tissue of the pancreas.

## SUMMARY

A study was made of the distribution of $Co^{60}$ in various tissues and organs of normal and alloxan diabetic rats during the first 24 hours following injection. No significant statistical differences were observed between the two groups of animals. Radioautographs of the pancreas in the two groups failed to provide any evidence that the cobalt in this organ might be concentrated in the islets of Langerhans.

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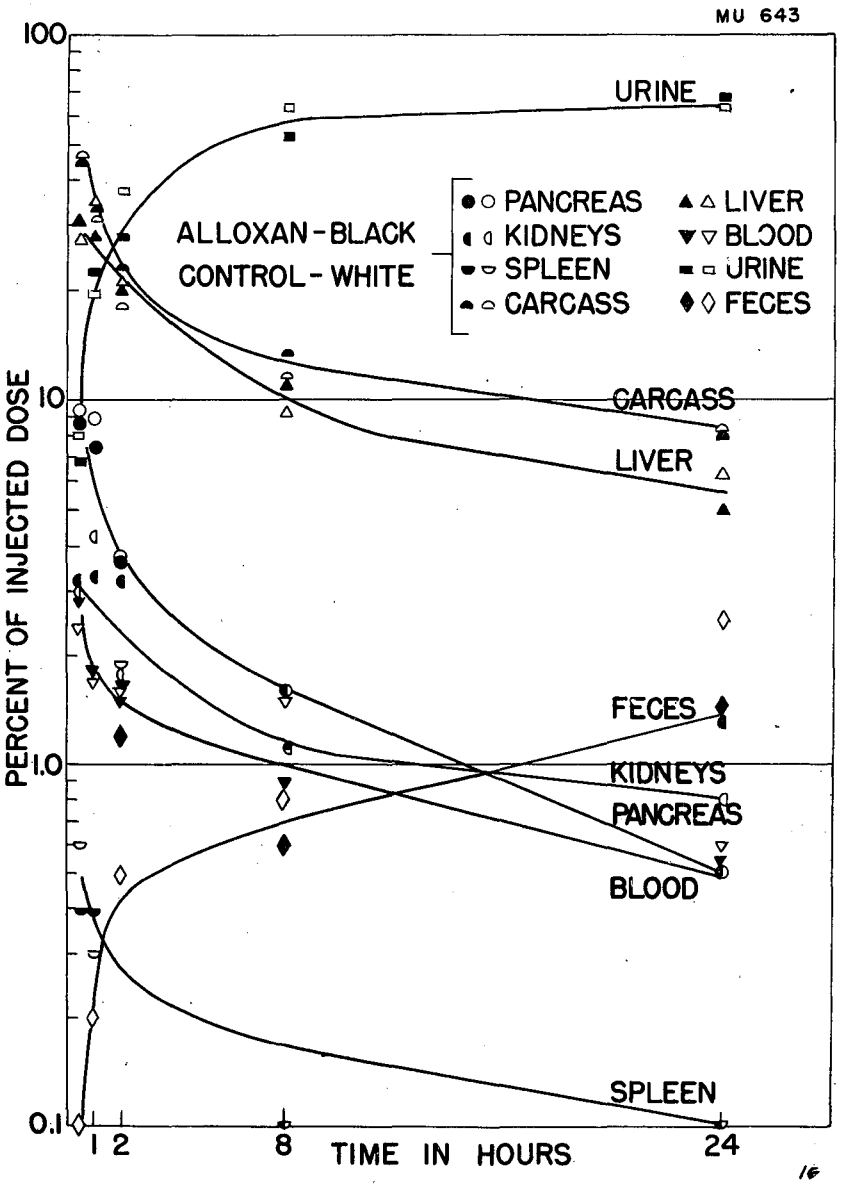


Figure 1. The distribution of intraperitoneally injected $Co^{60}$ in the tissues and excreta of normal and alloxan diabetic rats.

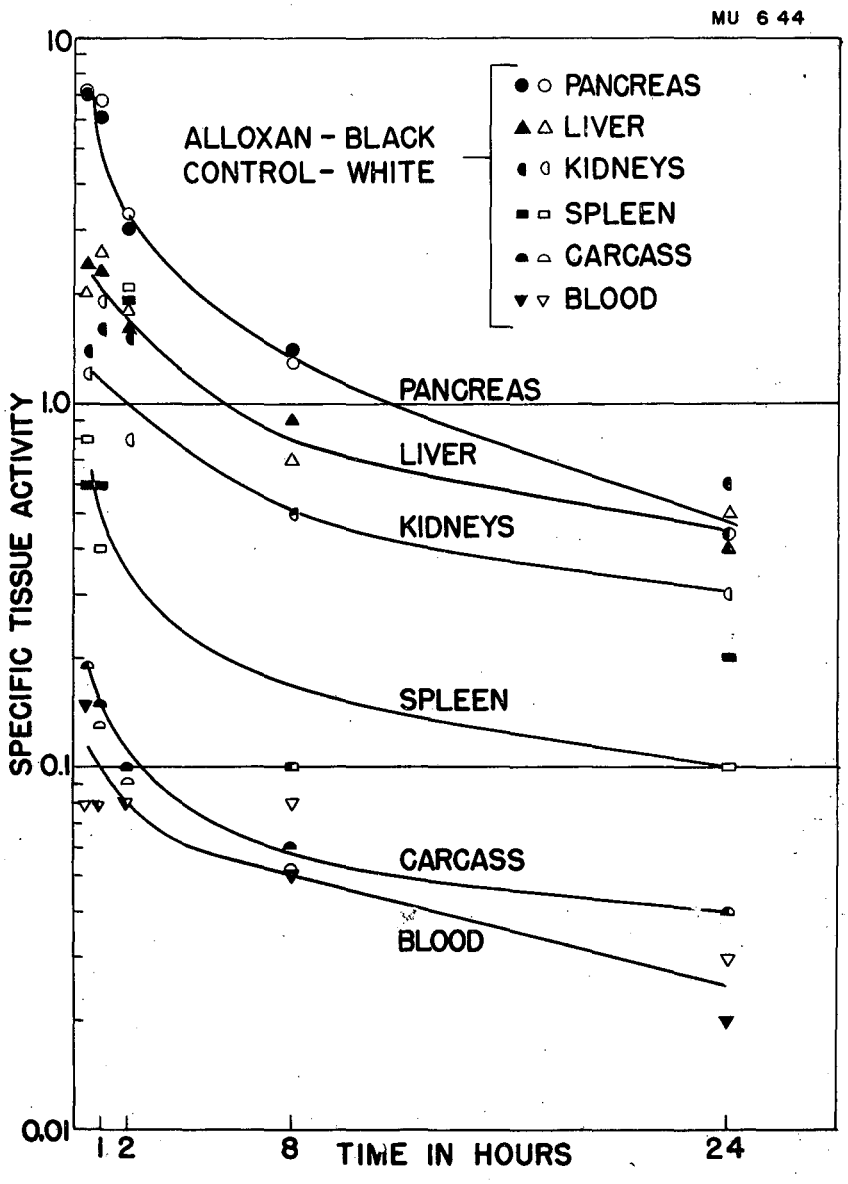


Figure 2. The specific $Co^{60}$ activity of various tissues in normal and alloxan diabetic rats.