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ALPHA CELL ACTIVITY AND ITS RELATION TO GLYCAEMIC LEVEL AND INSULIN SENSITIVITY IN ALBINO RATS.

(A COMPARATIVE STUDY OF THE ACTION OF NADISAN AND COBALT CHLORIDE).

BY

S. K. MUKHERJEE,

U. N. DE,

AND

B. MUKERJI.

(*From the Central Drug Research Institute, Lucknow.*)

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INTRODUCTION.

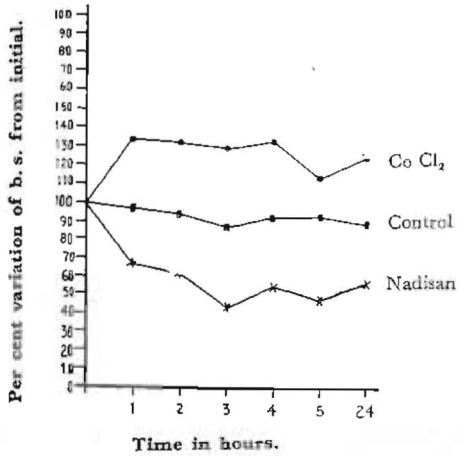
THE alpha cells of the islands of Langerhan's is of unknown functional significance and as such has been a matter of speculation during the last three decades. Experiments performed by Collip (1923), Kimball and Murlin (1923) and Burger and Kramer (1929, 1930) clearly showed that various commercial preparations of insulin contained a hyperglycemic and glycogenolytic principle, present therein as an impurity. This substance named glucagon was isolated and crystallized by Staub, Sinn and Behrens (1953, 1953*a*). The concept, that the source of this new hormone is the alpha cell of the pancreatic islands, was advanced by Heard *et al.* (1948), Sutherland and de Duve (1948), Audy and Kerly (1952), de Duve (1953), and Foa (1954).

That alpha cells of the pancreatic island is the source of glucagon was further demonstrated by Van Compenhout and Cornelis (1951, 1951*a*) and Van Compenhout *et al.* (1954) who produced selective destruction of the alpha cells in guinea-pigs by the use of cobalt chloride. Goldner, Volk and Lazarus (1952), Volk, Lazarus and Goldner (1953) and Lazarus, Goldner and Volk (1953) observed identical effect of cobalt chloride on the alpha cells of other species of animals.

That cobalt chloride not only caused definite structural change, but produced profound functional derangement of the alpha cells was demonstrated by Vuylsteke,

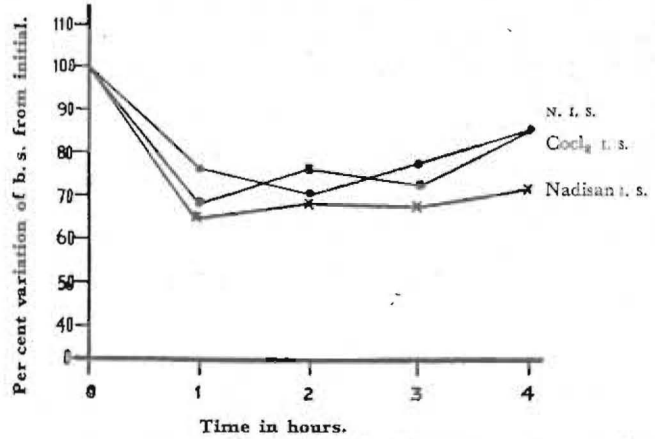
The effect of a single dose of Nadisan (·5 g./kg./oral) and cobalt chloride 50 mg./s.c. is shown in Graph 1. In Nadisan-fed rats the mean blood sugar level

GRAPH 1.



Effect of Nadisan (0·5 g./kg./oral) and cobalt chloride (50 mg./kg./S.C.) on the blood sugar level of normal rats fasted for 12 hours. Each point in the graph represents mean value of 6 observations. Co Cl₂ = cobalt chloride.

GRAPH 2.



Showing sensitivity to insulin (0·3 u/kg./S. C.) of Nadisan and cobalt-chloride treated rats fasted for 12 hours. Each point in the graph represents mean value of 6 observations.
N.I.S. = Insulin sensitivity of normal control.
$CoCl_2$ I.S. = Insulin sensitivity of cobalt chloride treated rats.
Nadisan I.S. = Insulin sensitivity of Nadisan treated rats.

recorded is 34 per cent below the initial level at the end of 1 hour. A maximum lowering of 57 per cent is reached at the end of 3rd hour. On the 4th hour, a secondary rise in blood sugar is observed which declines to 53 per cent on the 5th hour but at the end of 24 hours blood sugar is still below the initial level.

Cobalt chloride, on the other hand, has raised the blood sugar of all the animals up to the third hour after its injection. Mean values of percentage variation during 1st, 2nd, 3rd. 4th and 5th hours after the injections are $35 \pm 8{\cdot}64$; $32 \pm 7{\cdot}91$ and $30 \pm 4{\cdot}82$; $33 \pm 15{\cdot}13$; $14 \pm 9{\cdot}08$ i.e. roughly 30 per cent above the initial blood sugar level up to the 4th hour. Mean percentage variation of blood sugar 24 hours after the injection is $25 \pm 12{\cdot}65$. Blood sugar changes in normal fasting rat varied within —10 per cent during 24 hours period of observation.

The result of insulin sensitivity of cobalt-chloride treated and normal controls are comparable except that in the cobalt-chloride treated rats the maximum percentage variation of blood sugar ($-32 \pm 4{\cdot}51$) is recorded in the first hour instead of that in the second hour in the insulin-treated controls. On the fourth hour after insulin administration the percentage variation of blood sugar is the same (—15 per cent) in both the groups. In the Nadisan treated rats maximum percentage variation in the blood sugar is recorded on the first hour after insulin and is highest of all the three groups ($-35 \pm 3{\cdot}35$) and remains below —30 per cent all throughout the period of observation. On the 4th hour after insulin, it is still below —30 per cent, i.e. there is practically no recovery from the insulin hypoglycemia.

No variation in the liver glycogen value is observed as a result of Nadisan or cobalt-chloride administration. But a slight and a definite increase in the liver-cholesterol values are found in Nadisan and cobalt-chloride treated rats compared to the normal controls (Table III).

TABLE III.

Mean values of liver glycogen and cholesterol of cobalt chloride and Nadisan-treated albino rats.

Group.	Number of rats.	Liver glycogen* per cent.	Liver cholesterol mg./100 g.
Cobalt chloride treatment for 15 days	6	$3{\cdot}26 \pm 0{\cdot}48$	$634 \pm 52{\cdot}49$
Normal control ...	6	$2{\cdot}71 \pm 0{\cdot}3$	$264 \pm 18{\cdot}33$
Nadisan treatment for 43 days	6	$3{\cdot}31 \pm 0{\cdot}23$	$305 \pm 5{\cdot}86$

*Expressed as g. of glucose per 100 g. of wet tissue.

DISCUSSION.

It is evident from the results that prolonged daily administration of Nadisan cannot materially alter the fasting blood sugar level in albino rats except during the first three weeks. The apparent lowering of the blood sugar level after one

week of Nadisan feeding is possibly due to initial stimulation of insulin-secreting beta cells (Loubatieres 1944, 1944*a*, 1946 and Loubatieres *et al.*, *loc. cit.*).

It is difficult to explain the cause of increase in the blood sugar level in the 2nd and 3rd weeks after Nadisan treatment. It may be due to reaction to the hypoglycemic stress produced by Nadisan. Similarly, preliminary rise in blood sugar was observed by Chen *et al.* (1946), while using another hypoglycemic sulphonamide. Increase in the blood sugar level caused by cobalt-chloride injection is a non-specific phenomenon and is reproducible with every fresh injection of cobalt chloride. This phenomenon is quite distinct from the hyperglycemia caused by secretion of glucagon due to alpha cell stimulation by cobalt chloride (Goldner *et al.*, *loc. cit.*; Volk Goldner and Lazarus, *loc. cit.*; Foden and Reed, 1954; Koch, 1955). So, increase in the blood sugar level in some of the animals of cobalt-chloride treated groups can be explained on the above theory, or it may be due to a direct effect of $CoCl_2$ on the process of glycogenolysis (Volk, Goldner and Lazarus, *loc. cit.*). Our observation, however, fails to confirm this finding as no depletion of liver glycogen is observed by us (Table III).

The acute effects of cobalt-chloride injection and Nadisan feeding are in agreement with earlier workers who also found increase in the blood sugar level following $CoCl_2$ injection and lowering of blood sugar level following Nadisan feeding (Franke and Fuch, *loc. cit.*, Achelis and Herdeheck, *loc. cit.*; Bertram, Benfeld and Otto, *loc. cit.*; Volk, Lazarus and Goldner, *loc. cit.*; Lazarus, Goldner and Volk, *loc. cit.*; Van Compenhout and Cornelis, *loc. cit.*).

The more significant observation in this investigation is the nature of the insulin sensitivity in different groups of rats. We find that a definite difference of the insulin sensitivity exists in rats which have undergone prolonged treatment with two compounds both of which are noxious to the alpha cells. A months feeding of Nadisan in 0·5 g./kg./oral, daily or injection of $CoCl_2$ in 50 g./kg./s.c. daily, may be regarded to be sufficient to cause chemical paralysis of alpha cells. But under a similar circumstance, difference in the sensitivity to insulin is observed in the three groups of animals. The hypolgycemic level reached is more or less the same in all the three groups. In the normal controls and in the cobalt-chloride treated groups blood sugar showed earlier tendency to return to the original level. In the Nadisan-treated group hypoglycemia is prolonged and blood sugar is maintained almost at the lowest level reached (about —30 per cent) throughout the observation period of 4 hours.

This suggests that the persistence of insulin hypoglycemia in Nadisan-treated rats is a separate phenomenon and is probably due to the inhibition of insulinase enzyme (Mirsky *et al.*, 1956) or due to block at any other enzyme level concerned for glycogenolysis in the liver (Vaughan, 1956).

Liver-glycogen studies show that no depletion of liver glycogen has taken place following $CoCl_2$ or Nadisan treatment. There is slight change in the total cholesterol value of the liver in Nadisan-treated rats. These observations demonstrate that no toxic effect is exerted by Nadisan or cobalt chloride on the liver. Increase in the liver cholesterol in $CoCl_2$ treated animals is explained by the absence of alpha factor, which probably controls cholesterol metabolism (Caren and Carbo, 1956). Finally, it can be suggested that, apart from the alleged

chemical paralysis of alpha cells, Nadisan, in all probability acts at some enzymic level in the liver, and thereby directly or indirectly potentiates the action of insulin on carbohydrate metabolism.

Summary.

1. Nadisan feeding produced a profound fall in the blood sugar level of fasting albino rats. On prolonged administration, however, practically no variation in the fasting blood sugar level was observed after preliminary variations.

2. Subcutaneous cobalt chloride injection induced hyperglycemia in fasting albino rats, which lasted for 24 hours. On repeated subcutaneous injection, the fasting blood sugar level was raised in few rats.

3. Compared to normal and $CoCl_2$ treated rats, Nadisan treated rats, were more sensitive to insluin, as hypoglycemia produced thereby was prolonged.

4. No appreciable change in the liver glycogen was found in cobalt chloride and Nadisan treated rats. Liver cholesterol value was definitely increased following prolonged cobalt chloride injection.

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CHANGES IN VISCERAL AND METABOLIC ACTIVITIES AFTER FRONTAL AND TEMPORAL LOBE LESIONS*.

BY

B. K. ANAND†,

S. DUA,

AND

G. S. CHHINA.

(*From the Department of Physiology, Lady Hardinge Medical College, New Delhi.*)

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Recent physiological investigations have suggested that certain structures in the frontal and temporal lobes of the brain, included in the limbic system, are involved in the higher control of autonomic nervous system (MacLean, 1949; Fulton, 1953). As these regions have a marked influence over visceral activities, they have been collectively designated as the 'visceral brain'.

As a result of electrical stimulations of these regions carried out in unanæsthetized animals, Anand and Dua (1956) have already reported changes in the circulatory and respiratory responses, in blood sugar content (1956 *c*), in the gastric activity (1956 *d*), and other autonomic activities (1956 *a*, *b*). Surgical and electrolytic lesions involving the frontal and temporal lobes have now been produced and their effects on various visceral activities studied.

Material and method.

Frontal and temporal lobe lesions were produced in twenty-eight monkeys (*macacus*) and sixteen cats, out of which twenty-six monkeys and ten cats survived the operation, and only their results are reported.

In ten monkeys and four cats, bilateral surgical lesions involving large areas of the frontal lobes were made by aspiration after cauterizing their blood vessels. In three monkeys and one cat such lesions were made in the temporal lobes. The approaches were made through big craniotomy holes and bitemporal lobectomies achieved in two stages.

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†Present address.—Department of Physiology, All-India Institute of Medical Sciences, New Delhi.