# THE EFFECT OF ULTRA LENTE INSULIN ADMINISTRATION IN RATS

*By*

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Administration of Ultra Lente Insulin to male rats caused an increase of blood pressure and an enhanced response to the pressor action of angiotensin in a significant number of animals. The diuresis after a water load as well as the excretion of sodium and potassium in the urine was increased in these animals. This enhanced diuresis was prevented by administration of methyl dipyridyl propanone.

As Ultra Lente Insulin is very commonly used in the treatment of diabetes, a detailed study of the effects of chronic administration of Ultra Lente Insulin has been undertaken. This study was undertaken on 120 rats.

## METHODS AND MATERIALS

Male albino rats (100-130 gms.) were injected with 2.0 I.U. of Ultra Lente Insulin subcutaneously on alternate days for a period of 40 days. The following parameters were studied.

1. Blood pressure of the rats under urethane anaesthesia and sensitivity to exogenous Noradrenaline and Angiotensin was studied.

2. Diuresis after a water load (5 ml./100 gm. body weight) and the time to rate of maximal excretion was followed in the animals for three hours.

3. The weights of the heart, kidney, liver, adrenal and spleen were determined as a percentage of body weight in control and insulin treated animals.

4. The cholesterol level of adrenals, kidney and liver was measured after the method of Schoenheimer and Sperry (1934).

5. The Na and K content of adrenals and kidneys was measured after Leonard (1962).

6. The Na and K level in the urine and blood was estimated by means of Baird Atomic flame photometer.

7. The blood uric acid, cholesterol and haemoglobin levels were estimated.

8. Methyl dipyridyl propanone administration. In one group of animals methyl dipyridyl propanone was administered at a dose of 50 mg. (I. P.) daily for forty days together with the Ultra Lente Insulin. The blood pressure and the diuretic response to a water load was studied in these animals.

9. The blood sugar level in the Control and Insulin treated animals was estimated by the method of Johanson (1954).

10. Histopathological studies were performed on the liver, kidney, heart, adrenal, spleen and aorta of all animals after 40 days of Insulin treatment in these experiments.

The methyl pyridyl propanone used was Metopyrone( CIBA Ltd.)
The angiotensin used was Hypertensin (CIBA Ltd.)
The Ultra Lente Insulin used was from Dumex Ltd.

## RESULTS

*Blood Pressure*

The distribution pattern of the blood pressure in normal and insulin treated rats has been shown in fig. 1. It can be seen that the treatment with insulin tends to shift the average value to the higher side. It was also observed that in a

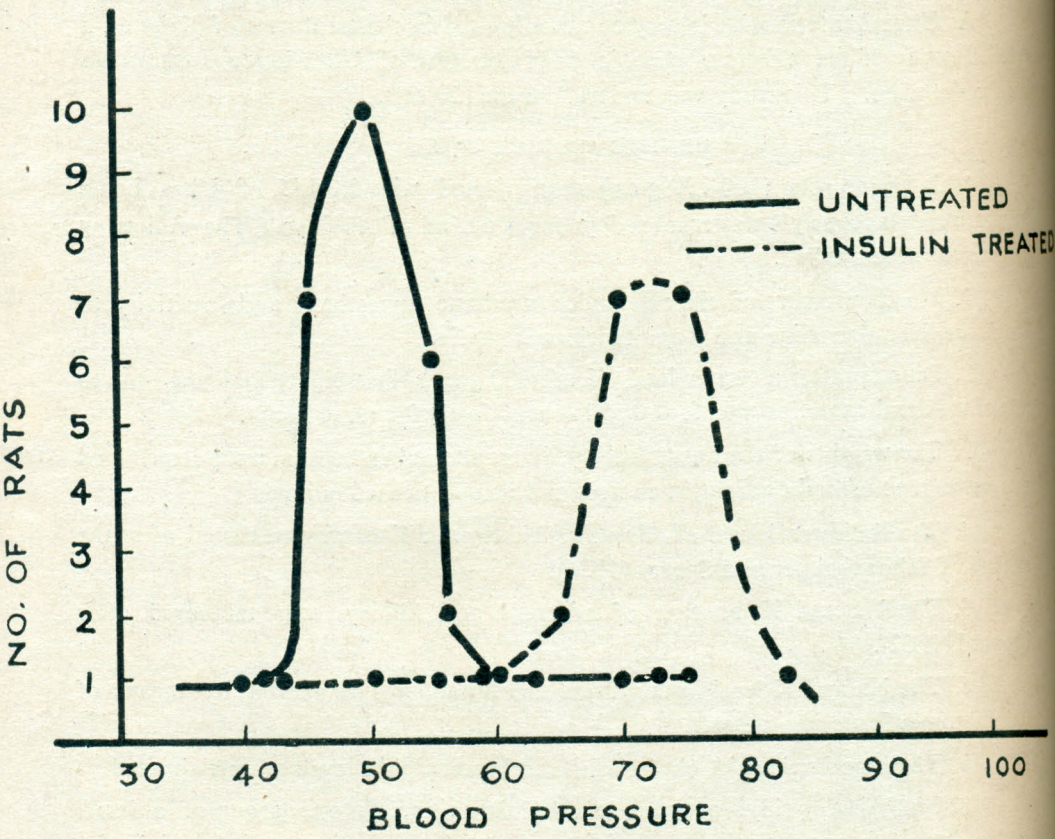


Fig. 1. Distribution pattern of the blood pressure of normal and insulin treated rats.

large number of insulin treated animals the pressor response to 0.1 microgram of angiotensin was exaggerated (Table 1). Figure 2 shows the blood pressure rise in response to angiotensin and noradrenaline in a normal and an insulin treated animal.

TABLE I

*The Pressor Effect of Angiotensin and Noradrenaline on the blood pressure of normal and Insulin treated rats.*

| | Angiotensin 0.1 Microgram. | | Noradrenaline 0.1 Microgram. | |
|---|---|---|---|---|
| | Control | Insulin treated | Control | Insulin treated |
| Mean rise of B. P. (mm. Hg.) | 13.2 | 20.6 | 8.2 | 8.1 |
| S. E. | = ± 1.57 | = ± 1.47 | = ± 0.61 | = ± 0.8777 |
| No. of rats used | 15 | 16 | 15 | 16 |

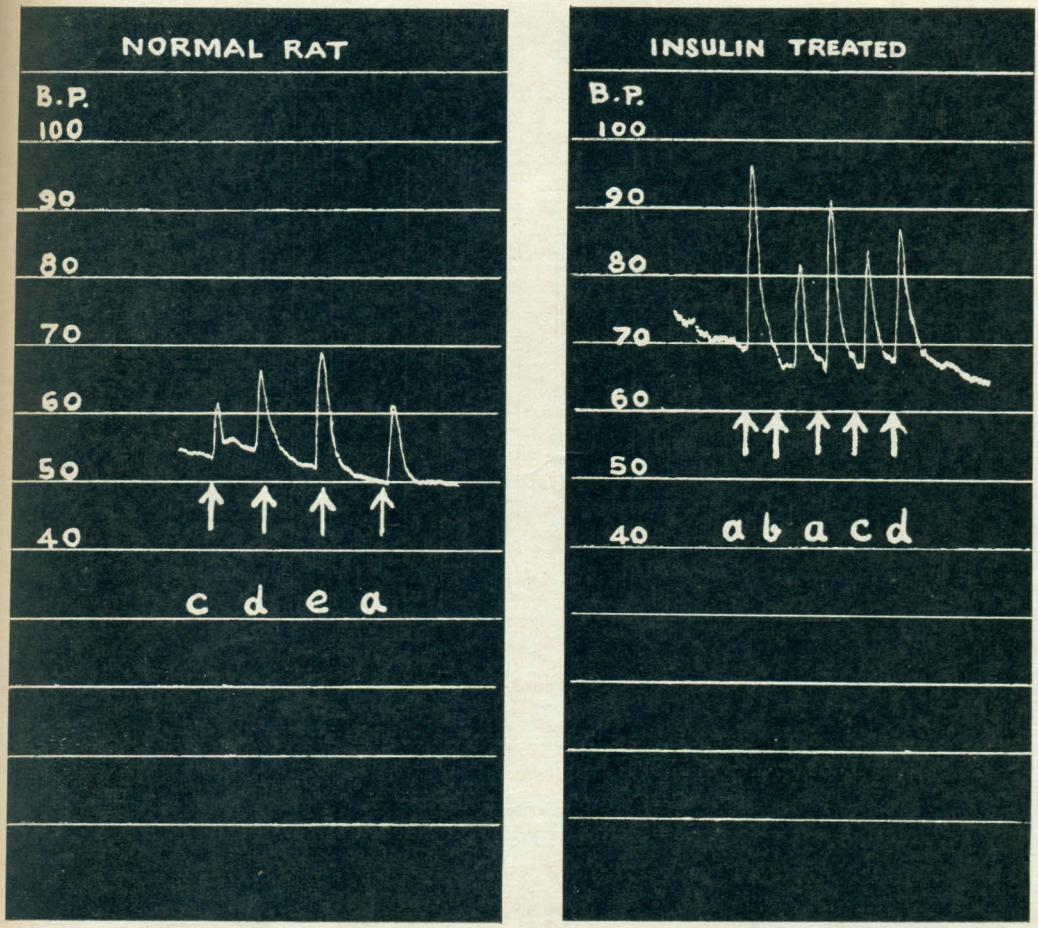


Fig. 2. The Pressor Effect of Angiotensin and Noradrenaline in the insulin treated and the normal rat.

a=0.1 μg Angiotensin b=0.05μg Angiotensin c=0.1 μg Noradrenaline
d=0.2 μg Noradrenaline e=0.2 μg Angiotensin.

The administration of methyl dipyridyl propanone did not prevent the raised blood pressure caused by the insulin administration as can be seen in fig. 3.

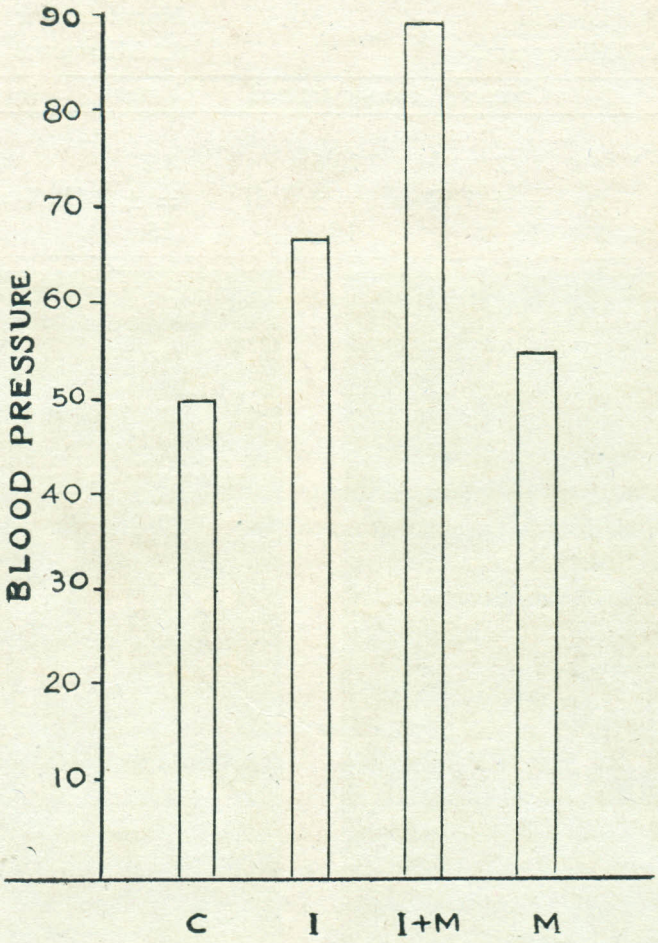


Fig. 3. The blood pressure of the rat after treatment with insulin and methyl dipyridyl propanone (in mm. of Hg.)
C=Control, I=Insulin treated,
M=Methyl dipyridyl propanone treated.

*Diuresis after a water load*

It was seen that in all experiments the insulin treated animals excreted out more rapidly a higher percentage of water than control animals. Table 2 shows the results in a typical experiment. The results of all the experiments are summarized in fig. 4. It can also be seen that the administration of methyl dipyridyl

TABLE II

*Percentage Diuresis after a Water Load of 2.5 ml/100 gm. bodyweight (0.25% NaCl)*

| | 2 Hours | 3 Hours |
|---|---|---|
| Control *Animals* | 30%, 26%, 28%<br>23%, 42.5%, 47%<br>29%, 35%, 59%.<br>Mean = 35.5% | 30%, 26%<br>28%, 23%, 42.5%<br>47%, 53%, 35%, 59%.<br>Mean = 38.1% |
| Insulin Injected *Animals* | 65%, 56%, 62%, 71%<br>84%, 76%, 94%, 87%<br>65%, 115%.<br>Mean = 77.5% | 65%, 88%, 62%, 71%<br>84%, 76%, 94%, 87%<br>65%, 115%.<br>Mean = 81.7% |

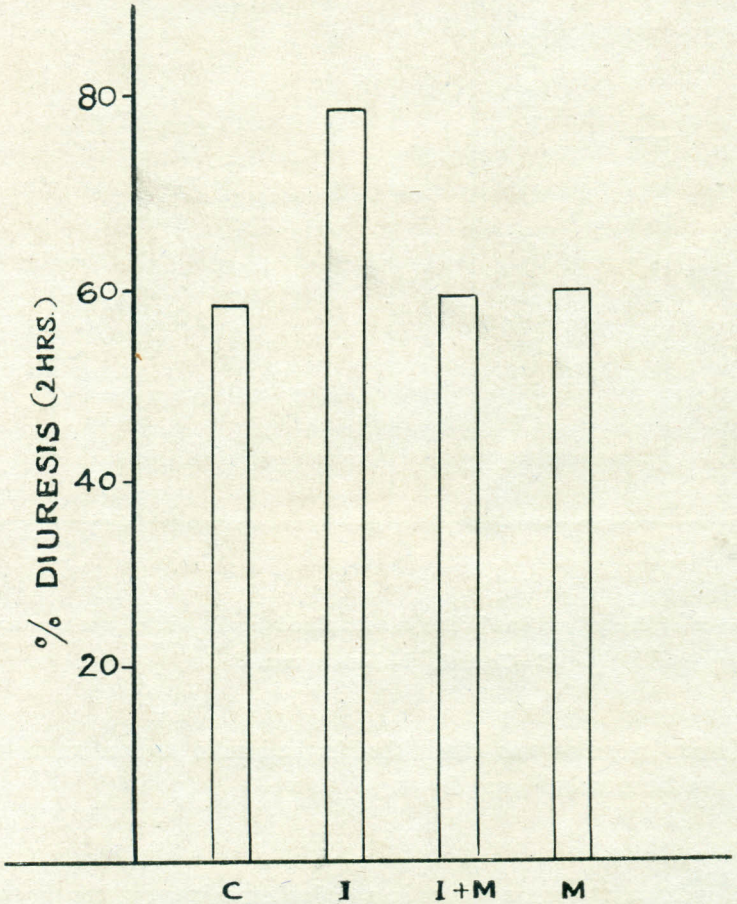


Fig. 4. The Diuresis after a Water Load in Rats treated with Insulin and methyl dipyridyl propanone.

C=Control, I=Insulin treated,
M=Methyl dipyridyl propanone.

propanone prevented this enhanced diuresis after a water load. Figure 5. depicts the excretion of sodium and potassium in the urine after a water load. There was enhanced excretion of sodium and, to a lesser extent, of potassium. The time to

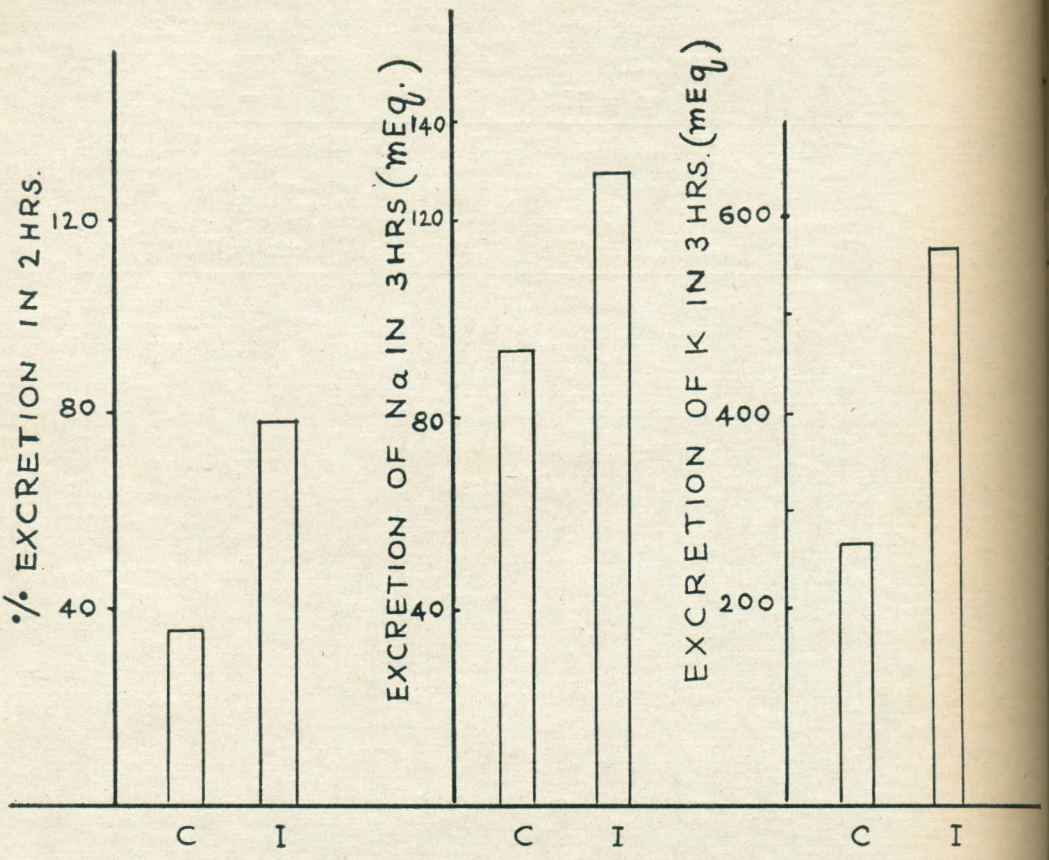


Fig. 5. Excretion of Electrolytes and Water in normal and insulin treated rats after a water load. C=Control, I=Insulin treated.

rate of maximum excretion was diminished in the insulin treated animals and the mean values can be seen in Figure 6.

*Tissue weights*

Table 3. indicatest he mean values in a typical experiment where the tissues have been weighed in control and insulin treated animals. There was no difference in the weights of any organ except that the spleen weight decreased in the insulin treated animals to a significant extent ($P = \leqslant 0.01$).

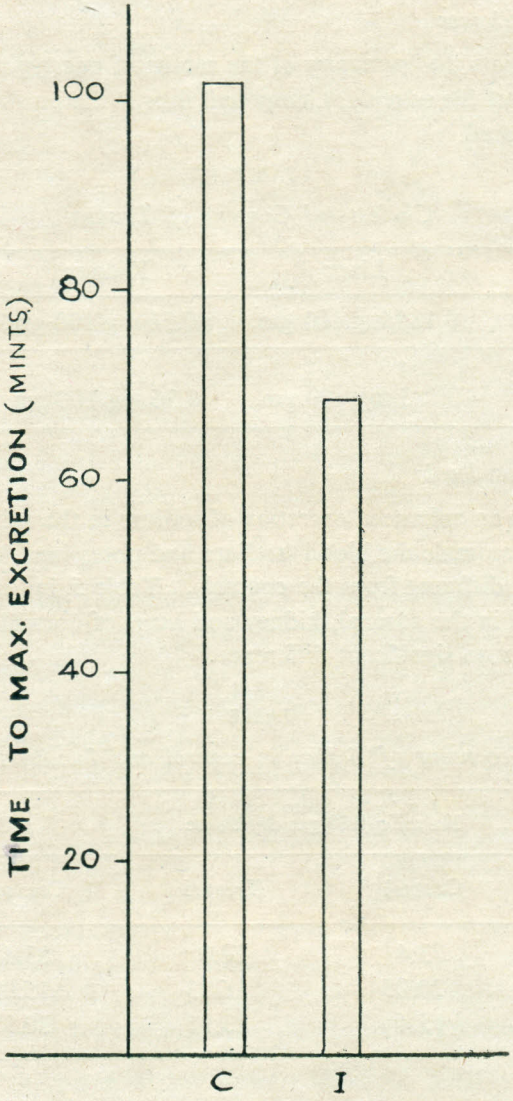


Fig. 6. Time to the rate of maximum excretion in normal and insulin treated rats after a water load.

C=Control, I=Insulin treated.

TABLE III

*Mean Organ Weights.*

| | Liver | Kidney | Heart | Spleen | Adrenal |
|---|---|---|---|---|---|
| Controls | 3.22 gm/ 100 gm. | 0.55 gm/ 100 gm. | 0.27 gm/ 100 gm. | 0.17 gm/ 100 gm. | 9.8 mg/ 100 gm. |
| Insulin treated animals | 3.30 gm/ 100 gm. | 0.54 gm/ 100 gm. | 0.29 gm/ 100 gm. | 0.14 gm/ 100 gm. | 10.8 mg/ 100 gm. |

*Cholesterol level of tissues*

Table 4 depicts the summary of the results of one typical experiment. The cholesterol content of the adrenal, kidney and liver in the insulin treated animals was not significantly altered.

TABLE IV

*Cholesterol Content of Tissues*

| | Liver | Kidney | Adrenal |
|---|---|---|---|
| Controls (10) | 0.188 mg/100 gm. | 0.31 mg/100 gm. | 2.95 mg/100 gm. |
| Insulin treated animals (10) | 0.183 mg/100 gm. | 0.36 mg/100 gm. | 3.46 mg/100 gm. |

*Sodium and potassium levels*

As there was an enhanced excretion of sodium in the urine after hydration in the insulin treated animals the blood sodium and potassium levels were estimated but were in no way different from the controls. Table 5 indicates also the sodium and potassium level in the adrenal, kidney and heart of normals and insulin treated animals. There was no significant difference.

TABLE V

*Na and K content of tissues in control and Insulin treated rats*

| Tissue | Na mEq./100 gm dry tissue | | K mEq./100 gm dry tissue | |
|---|---|---|---|---|
| | Control | Treated | Control | Treated |
| Adrenals | 23.4 | 27.0 | 83.4 | 84.0 |
| Kidneys | 20.05 | 16.13 | 15.09 | 20.15 |
| Heart | 15.4 | 13.91 | 30.93 | 30.76 |

*Blood examination*

Table 6 shows the results of a typical experiment where the blood haemoglobin, uric acid and cholesterol levels have been compared in normal and insulin treated animals. Although all the three values are higher in the insulin treated animals, the difference is not statistically significant.

*Miscellaneous studies*

The blood sugar levels were measured in the insulin treated animals and these were in no way different from the blood sugar level in control animals. There was no hypoglycemia present even after forty days of treatment.

TABLE VI

*Blood Uric acid, Cholesterol and Haemoglobin values in control and Insulin treated rats*

| Component | Control | Insulin treated |
|---|---|---|
| Uric acid mg/100 cc. | 4.90 | 5.76 |
| Cholesterol mg/100 cc. | 88.7 | 112.5 |
| Haemoglobin gm/100 cc. | 14.57 | 16.11 |

*Histopathology*

Histopathological examination of the kidney, liver, heart, aorta, adrenal and spleen of the insulin treated animals revealed no outstanding pathological changes which could account for the pharmacological findings.

## DISCUSSION

It was found that a large proportion of the rats receiving Ultra Lente Insulin injections developed a higher basal blood pressure than control animals. This increase in the blood pressure was not prevented by administration of methyl dipyridyl propanone which did, however, block the enhanced diuretic response seen in these rats after a water load. Since methyl dipyridyl propanone blocks beta hydroxylation (Brownie, 1962) this enhanced diuresis in the insulin treated animals could have been due to increased adrenocortical activity. The time to rate of maximal excretion was also markedly diminished in the insulin treated animals.

Another cause of the increased blood pressure could be the enhanced sensitivity of the blood vessels to angiotensin and noradrenaline. Our results indicate that angiotensin administration caused a greater pressor effect in insulin treated animals than in normals and this increased sensitivity to angiotensin could be, in part, responsible for this type of experimental hypertension.

Insulin might bring about some degree of atherosclerosis because of its anabolic effect on lipid metabolism (Chain, 1959) and so the cholesterol content of the blood and certain tissues were estimated. There was no increase in the cholesterol content in blood or in the adrenal, liver or kidney and no evidence was therefore obtained to substantiate this hypothesis.

The results obtained from the electrolyte studies are interesting. After a water load there was increased excretion of sodium and potassium. The increased water excretion was probably caused by the excretion of the sodium. However, the blood level of the electrolytes and also the tissue electrolyte levels in the adrenal, kidney and heart were not raised.

The blood studies indicate that the haemoglobin level was increased and this could be the result of a high resting blood pressure. The increase in the uric acid level was not significant.

A clear involvement of the adrenals in this type of experimental hypertension could have been demonstrated by trying to produce this insulin hypertension in adrenalectomized animals. This was attempted but the adrenalectomized animals did not survive the administration of insulin and so no conclusions can be drawn. It is possible that there may be factors other than increased sensitivity of the blood vessels to angiotensin and enhanced adrenocortical activity which contributed to the high blood pressure in the insulin treated animals.

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