THE EFFECT OF FASTING, COLD STRESS AND ACTH ADMINISTRATION ON THE BLOOD SUGAR AND LIVER GLYCOGEN LEVELS OF NORMAL AND ADRENALECTOMISED RATS AND MICE

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Fasting or exposure to cold lowers the blood sugar and liver glycogen levels of rats and mice. These decreases are not affected by adrenalectomy but they are retarded by pretreatment with ACTH.

THE rôle of the adrenal cortex in carbohydrate metabolism is not clear, although it has been known for a long time (Britton, 1931) that carbohydrate changes follow adrenalectomy. Britton and his colleagues (Britton and Silvette, 1932; 1934; Britton, Kline and Silvette, 1938; Britton, Silvette and Kline, 1938a,b) concluded that death after adrenalectomy was due to a failure of carbohydrate metabolism. Parkins, Hay and Swingle (1936), however, considered that adrenalectomy led to no disturbance of carbohydrate metabolism. Further, in 1937, Deuel, Hallman, Murray, and Samuels reported that, if rats were maintained on sodium chloride, glycogen deposition in the liver was not impaired by adrenal insufficiency.

Long, Katzin and Fry (1940), in a review of the influence of the adrenal cortex on carbohydrate metabolism, agreed with Deuell and others that adrenalectomised rats and mice maintained on sodium chloride and glucose had no abnormalities of carbohydrate metabolism; when fasted, however, a rapid fall of liver and muscle glycogen occurred.

Animals become more susceptible to stress after adrenalectomy. For example, adrenalectomised mice exposed to cold die more quickly than sham-operated controls. Further, D'Arcy (1957) showed that administration of adrenocortical hormones enabled adrenalectomised mice to survive in the cold as long as, or if the dose was sufficiently high, even longer than, untreated intact mice. A number of adrenal steroids possessing glucocorticoid activity gave this protection, whereas deoxycortone was relatively ineffective (D'Arcy, 1956). Some workers, like Winternitz, Dintzis and Long (1957), have found that the liver glycogen is lowered by adrenalectomy, whilst others, like Young (1946) state that it is unchanged. However, workers agree that the liver glycogen and blood sugar levels of adrenalectomised animals fall rapidly when fasted.

The work reported here was undertaken to examine the effect of adrenalectomy on the carbohydrate metabolism of rats and mice subjected to stress.

EXPERIMENTAL

Materials and Methods

Wistar rats and mice of the Swiss strain were fed on a cube diet and water, and maintained at a constant temperature of 70°F. Adrenalectomised animals were kept on a normal diet but given 0·9 per cent saline to

drink. Groups of 6 male rats (150–170 g.) and groups of 10 male mice (15–18 g.) were used.

A long-acting preparation of ACTH, Cortrophin Zn (Organon), was injected subcutaneously in volumes of 0·1 ml. (4 units) into mice and 0·5 ml. (20 units) into rats. Injections of normal saline were given in similar volumes to control animals.

Experimental Procedures

Adrenalectomy under ether anaesthesia was by the usual lumbar approach. Adrenalectomised mice were used 48 hr. after, and adrenalectomised rats 5 days after, removal of their adrenal glands. Fasted animals were deprived of food but allowed free access to water or saline. Exposure to cold was effected by placing the animals for varying times in a cold room maintained at 2 ± 0·5°. Mice were placed in pairs under inverted food hoppers and rats were kept in cages containing three animals. The

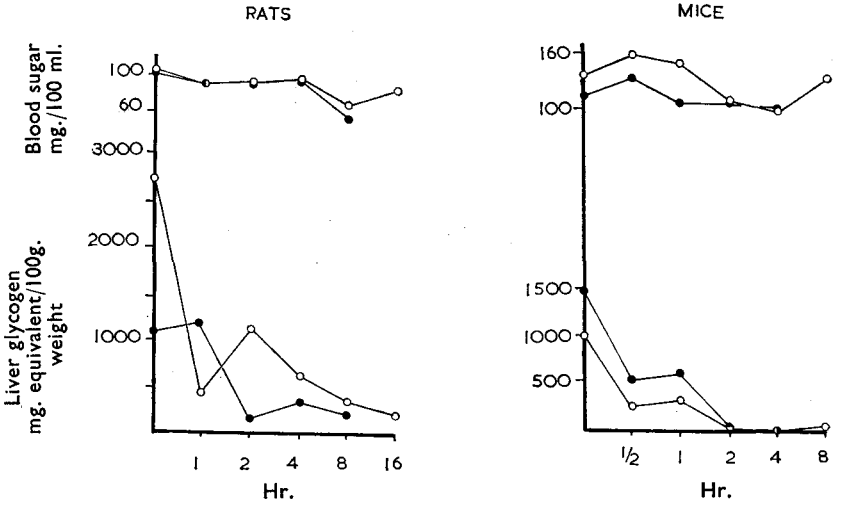

FIG. 1. The effect of cold stress on the blood sugar and liver glycogen levels of intact (○) and adrenalectomised (●) rats and mice.

floors of all the cages were covered with paper to absorb urine. Cold-stressed animals were killed, and their tissues removed, inside the cold room. ACTH was administered 15 hr. before commencement of fasting or of exposure to cold, and a similar dose of ACTH was given 24 hr. later if the animals survived. Liver glycogen was determined by the method of Block and D'Arcy (1958) and blood sugar by the method of Hagedorn and Jensen (1923).

RESULTS

The results of the experiments are summarised in Figs. 1–4.

Effect of Cold Stress and Fasting on Carbohydrate Levels

In both rats and mice, exposure to cold produced a rapid fall in the levels of liver glycogen. Although adrenalectomy did not alter the rate

of fall of liver glycogen, the adrenalectomised animals died earlier. For example, the adrenalectomised mice were dead after 8 hr. exposure and the adrenalectomised rats, with one exception, were dead after 16 hr.,

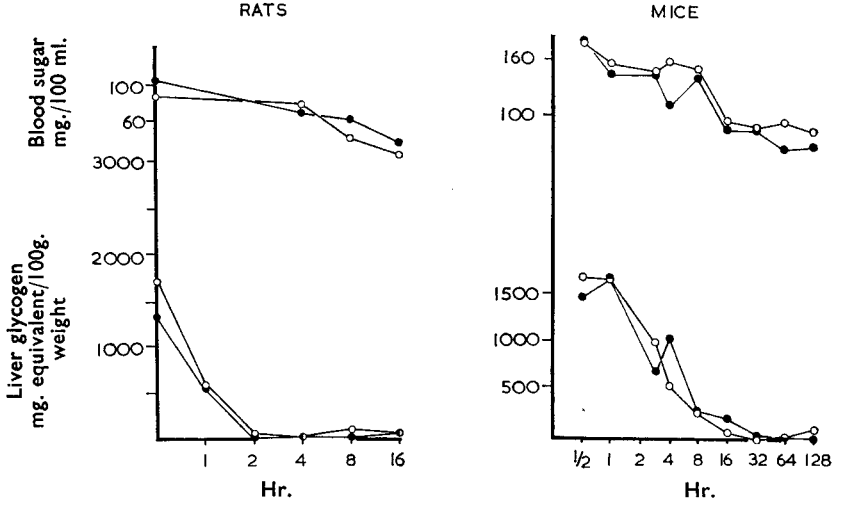

FIG. 2. The effect of fasting on the blood sugar and liver glycogen levels of intact (○) and adrenalectomised (●) rats and mice.

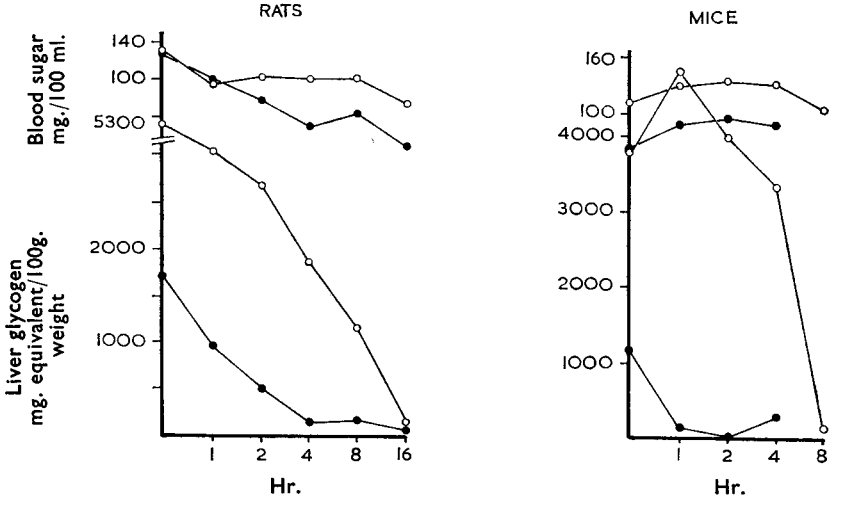

FIG. 3. The effect of ACTH administration on the blood sugar and liver glycogen levels of intact (○) and adrenalectomised (●) cold stressed rats and mice.

whereas the unoperated animals in each case were alive at these times. Fasting produced a similar fall of liver glycogen and a marked lowering of the blood sugar in both intact and adrenalectomised rats and mice.

Effect of ACTH *Treatment on Carbohydrate Levels After Cold Stress or Fasting*

ACTH administration had no effect on the liver glycogen levels in adrenalectomised animals whereas the levels in unoperated animals were markedly raised. For example, whereas the mean liver glycogen level in untreated mice was 1,500 mg. glycogen equivalent per 100 g. weight, after ACTH administration it was nearly 4,000; similarly, the level in untreated rats was less than 3,000, and after ACTH it was more than 5,000. Moreover, after fasting or exposure to cold, the raised levels remained high for a long period of time; for instance, after 4 hr. exposure to cold, the liver glycogen level in mice treated with ACTH was still more than 3,000

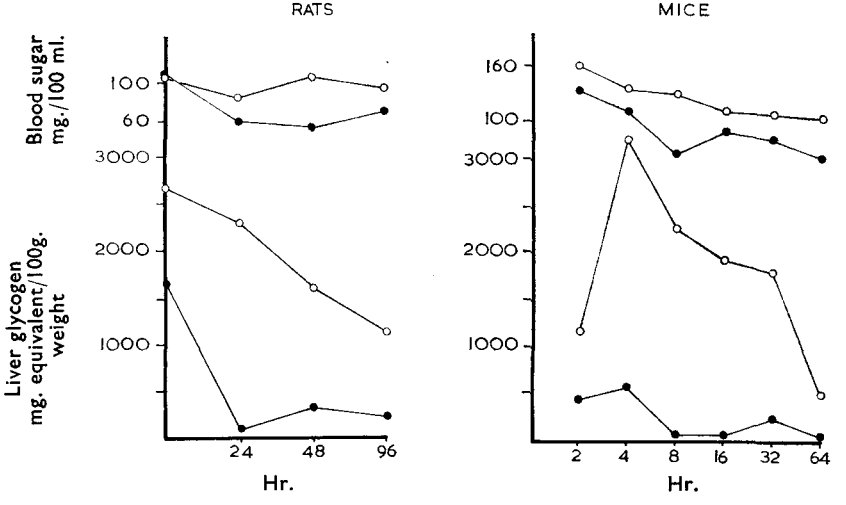

FIG. 4. The effect of ACTH administration on the blood sugar and liver glycogen levels of intact (○) and adrenalectomised (●) fasting rats and mice.

mg. equivalent glycogen per 100 g. weight whereas the level in the uninjected counterparts was almost zero. The blood sugar levels of the ACTH-treated unoperated animals of all four experiments were only slightly higher than those of the ACTH-treated adrenalectomised animals.

DISCUSSION

The Effect of Cold Stress

In this present work it has been shown that when rats and mice die as a result of cold stress, death is independent of the rate of fall of their carbohydrate levels. Death under these conditions is not, therefore, the direct result of failure of carbohydrate metabolism.

Pretreatment of the animals with a large dose of ACTH some hours before the onset of the stress resulted in some alterations in the carbohydrate picture. In unoperated animals the levels were elevated above the resting level but there was no change in adrenalectomised animals. After 8 hr. in the cold, the liver glycogen levels of unoperated mice plunged to a level

reached by the adrenalectomised mice in 1 hr. In the rats, a similar but somewhat less precipitous fall occurs. It is interesting that administered ACTH has this effect, whereas the secretion of animal's pituitary gland does not produce a similar retention of carbohydrate. It may be that the stress is not sufficient to evoke an increased output of ACTH; or that the amount of ACTH produced is not enough to stimulate the adrenal cortex; or that the adrenocortical hormones, even if stimulated by the pituitary, are not produced in a sufficient quantity to influence carbohydrate metabolism.

The protection against cold is produced only by large doses of cortisone, about 1·25 mg/kg. (D'Arcy, 1956). However much ACTH is present, and however great its stimulus, the amount of steroid produced is nevertheless limited by the secreting capacity of the adrenal cortex. This maximum secretion may be sufficient to have a marked effect on carbohydrate metabolism but be insufficient to protect against stress. It may be that corticosterone—the main adrenal cortical steroid in the rat and mouse, and cortisone have two effects; one, to increase the survival time under conditions of stress and the other to exert a carbohydrate effect. A further possibility is that the action on carbohydrate is the more easily produced and the extended survival only occurs when a larger dose is administered. When a large dose of ACTH is administered, the secretion of corticosterone is increased and so the carbohydrate levels rise. Similarly, the protective mechanism of cortisone in cold stress may be explained by the dose being so high that the property of life maintenance becomes apparent.

The Effect of Fasting

The blood sugar and liver glycogen levels of normal and adrenalectomised rats and mice fell rapidly and steadily after fasting. Not only did many mice survive, but the survivors were equally distributed between the unoperated and adrenalectomised groups. This result is at variance with those of Britton and his colleagues and shows that adrenalectomised animals do not die because of a failure of their carbohydrate metabolism. However, Cox (1957) showed that the blood sugar of adrenalectomised rats fell to a lower level than that of normal rats when they were subjected to fasting.

The results reported here do not agree with those of other workers. Long (1942), for example, stated that the removal of the food from adrenalectomised animals depletes the liver of its glycogen whereas that of normal animals is unaffected. Further, Evans (1941) reported that adrenalectomised animals lost more carbohydrate than did unoperated animals in the early hours of fasting. However, in the present experiments, the liver glycogen levels of both normal and adrenalectomised animals fell at the same rate after fasting. The fall of carbohydrate in intact animals is difficult to explain, as ACTH administration delays the fall. The endogenous adrenal steroids secreted without ACTH administration may be either incapable of converting protein into carbohydrate faster than carbohydrate is being utilised, or there is no gluconeogenesis. The

results obtained from the experiments with ACTH support the former possibility. The large amount of adrenal steroid secreted as a result of the ACTH stimulus possibly enables the rate of gluconeogenesis to exceed that of glycolysis. The fall of the carbohydrate levels towards the end of the fasting period could be due to the diminishing effect of the ACTH: although the ACTH preparation used is long acting for man, it is not necessarily so for rats and mice, for the metabolism of these animals is faster.

An investigation into the blood corticosterone levels under the conditions of the present experiments may shed more light on this problem.

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