THE EFFECT OF BARBITURATE ANÆSTHESIA ON THE BLOOD α-KETO ACID LEVELS IN RATS AND RABBITS

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Received December 5, 1951

During a recent investigation[1] into an alleged vitamin A-aneurine synergism[2] it was necessary to determine the blood α-keto-acid (pyruvate and α-ketoglutarate) levels in rats. It is not easy to obtain true resting values because the slightest degree of struggling of the animal results in a considerable increase in these acids.[3,4] To overcome this difficulty cord resection[5] and light barbiturate anæsthesia[6] have been recommended. The latter procedure appeared most suitable for our purpose, but before it was adopted the effect of the barbiturate to be used (pentobarbitone, nembutal) on blood keto-acid levels was investigated in detail. It was found that anæsthesia is accompanied by a considerable decrease in these blood constituents.

Experimental

Animals. (*a*) *Rats.* Adult rats (about 250 g.), previously deprived of food for 18 hr., were injected intraperitoneally with 0·1 ml. of a solution of pentobarbitone containing 1 grain (65 mg.) in 1 ml and held firmly to prevent struggling until anæsthesia was complete. Blood samples were drawn at appropriate times by opening up the thorax, cutting the aorta, and allowing the blood to flow into a small (10 ml.) beaker which had been previously moistened with citrate; this anti-coagulant is recommended because it tends to stabilise the keto-acids.[7] As soon as possible after drawing, aliquots of the blood were delivered into a known volume of trichloracetic acid and the pyruvate and α-ketoglutarate levels determined by a modification[1] of the Friedemann-Haugen method.[8]

(*b*) *Rabbits.* Adult rabbits (about 2·5 kg.), previously deprived of food for 18 hr., were injected intraperitoneally with 1·2 ml. of the solution of pentobarbitone and blood samples drawn at the appropriate time from a marginal ear vein. The blood keto-acid levels were examined in the manner just referred to.[1]

Results

(*a*) *Rats.* Figure 1 records the results obtained in an experiment involving 5 rats. As 2 ml. of blood is required for a duplicate determination of keto-acids, one animal has to be sacrificed for each determination; it is thus not possible to follow the change in levels in a single animal during narcosis; animals were therefore killed at various times after injection of the anæsthetic and the values obtained plotted on the curve reproduced in Figure 1. The resting levels were taken as those obtained as soon as anæsthesia was sufficiently advanced to allow the body wall to be opened; this was generally within 2 to 3 minutes of injection. The assumption

that this gives a true picture of the resting values 9·14 μg./ml. (S.D. ± 2·31) for pyruvate and 5·76 μg./ml. (S.D. ± 2·70) for α-ketoglutarate is justified because these mean values, obtained on 20 rats drawn at random from a stock colony, approximate very closely to the values reported by other workers[9,10] using different methods for obtaining a basal value.

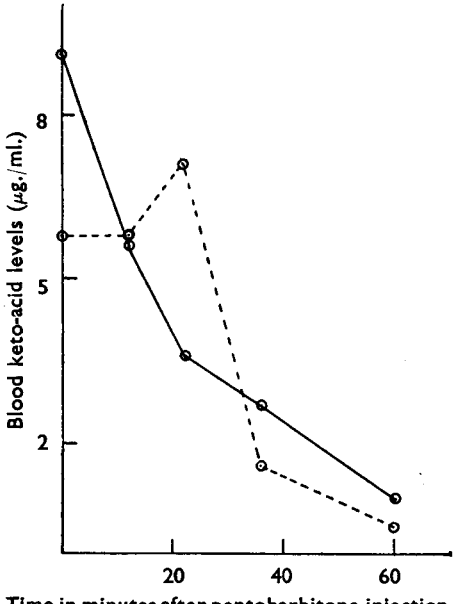

FIG. 1. The fall in the blood pyruvate and α-keto-glutarate levels of rats after intraperitoneal injection of pentobarbitone (0·1 ml.).

——— Pyruvate levels.
– – – – – α-Ketoglutarate levels.

It will be seen from Figure 1 that there is a steady fall in pyruvate levels until, 60 minutes after injection, there is almost no pyruvate in the blood. The α-keto-glutarate follows the same pathway; the slight increase noted 22 minutes after injection cannot be considered significant, when the normal variations (S.D. ± 2·70) are taken into account together with the fact that the accuracy of the determination of this keto-acid is less than that of pyruvate.

(*b*) *Rabbits.* The results obtained for the pyruvate levels of rabbits during anæsthesia are recorded in Figure 2. In this experiment the change in levels could be followed in the same animal, and it will be seen that the same general picture is obtained as with rats. The fall in pyruvate levels continues for 60 to 75 minutes after narcotisation, and 2 hr. afterwards the normal levels had not been restored, although the rabbits were recovering from the anæsthetic. It should be pointed out that the base-line levels for the rabbits were obtained before injecting the barbiturate; the values are somewhat higher than those previously recorded,[9] but in the present experiments no particular efforts were made to prevent the rabbits from struggling. The changes in the α-keto-glutaric levels of the rabbits are not recorded here, but, as in rats, they showed the same general downward tendency.

One experiment was carried out in which a rabbit was first injected with pentobarbitone and immediately afterwards with adrenaline (1 ml. of 1 in 500), which is known to raise blood keto-acid levels considerably.[11,12] 30 minutes later the blood levels of both pyruvate and α-keto-glutarate were about 75 per cent. above their original values; this was in spite of the fact that the animal was deeply anæsthetised.

Discussion

The results presented show quite clearly for the first time that blood pyruvate and α-keto-glutarate levels of rats and rabbits fall rapidly during anæsthesia produced by a typical barbiturate. This is the reverse of the effect observed in the case of blood lactate levels which have been found to rise on the administration of hexobarbitone[13] and thiopentone.[14] Until much more is known concerning the mode of action of barbiturates in tissues other than brain, especially on carbohydrate metabolism, neither the importance nor the significance of this drop in keto-acids can be fully assessed. It does seem, however, that as (*a*) levels are still normal immediately after narcotisation is complete and (*b*) anæsthesia can continue although the levels are above normal (as after the simultaneous administration of the narcotic and adrenaline), the fall in blood keto-acid levels is not directly concerned with either the onset or the maintenance of anæsthesia.

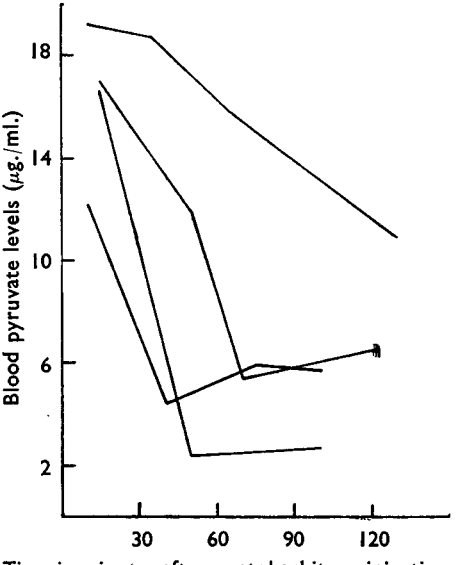

FIG. 2. The fall in the blood pyruvate levels of rabbits after intraperitoneal injection of pentobarbitone (1·2 ml.). Each curve represents a different animal.

The drop in pyruvate levels during narcosis may be caused by the existence of a sub-basal metabolic condition rather than by direct inhibition of glycolysis; the concomitant increase in lactate levels is probably due to inhibition of respiration, the smaller amount of pyruvate formed being reduced to lactate under the increased degree of anærobiosis prevailing, instead of being oxidised aerobically to carbon dioxide and water.

It may be presumed that the reduced levels of α-keto-glutarate in the blood are also caused by the lowered rate of carbohydrate catabolism which must accompany the general functional depression caused by the barbiturate.

Summary

1. The blood pyruvate and α-keto-glutarate levels of rats and rabbits fall rapidly during pentobarbitone-induced anæsthesia.

2. The fall can be counteracted by the simultaneous injection of adrenaline.

Our thanks are due to Prof. R. A. Morton, F.R.S., for his interest in this work, and to the Medical Research Council for a personal grant to one of us (G. R. W.) and for a grant towards laboratory expenses.

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