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CENTRAL NERVOUS FUNCTION AND CHANGES IN BRAIN METABOLITE CONCENTRATION: CHARACTERISTIC GLYCOGEN INCREMENT PATTERNS PRODUCED BY CONVULSANT DRUGS

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In a previous publication (Chance and Yaxley, 1950), it has been reported that a rise in brain glycogen occurred during convulsions in mice. The brain glycogen rose only when the precipitating stimulus was of sufficient strength to produce a convulsion. In this sense the glycogen increment was found to be specifically associated with convulsions. The amount of the increment was not only related to the intensity of extrinsic stimuli in normal mice at convulsive levels and above, but also was directly related to the severity of the convulsions. Moreover, this increment varied in the various different major subdivisions of the brain after a standard stimulus. It was therefore concluded that the glycogen increment in any part of the central nervous system could be taken as an indication of the amount of " nervous activity " arising from a particular region during convulsions. Seizures in man and animals show a variety of forms apart from a major change from a clonic to a tonic state with increasing severity. Similar differences exist between the patterns of convulsive movement produced by maximal doses of different central nervous stimulants and of other drugs producing convulsions. It was therefore thought worth investigating whether the patterns of glycogen increment would differ accordingly and be a reliable guide to the patterns of central nervous activity underlying the differences between the seizures. Thus the glycogen increment patterns produced by convulsive doses of a variety of substances, which are already well known for their action on the central nervous system at subconvulsive dose levels, have been plotted.

METHOD

Male mice of a single strain weighing 20–25 g. were used throughout. Individual animals were given the dose required to produce convulsions in 50 per cent of animals (CD50) in 10 ml./kg. body weight, by intravenous injection; they were killed at various and, whenever possible, comparable intervals of time after the injection, during the convulsion and sometimes up to ninety minutes after the convulsions. This procedure produced clonic convulsions in all those animals which convulsed. In this way the rate of rise of glycogen, which always reached its peak at the end of the convulsion, was assessed in different parts of the brain as the convulsion proceeded.

Drugs were dissolved in aqueous solution. The CD50 values, which had been previously determined on this strain of mice, were as follows: *dl*-amphetamine sulphate, 40 mg./kg.; sodium diphenyl-hydantoin, 60 mg./kg.; caffeine (alkaloid), 35 mg./kg.; nikethamide (*B.P.*), 50 mg./kg.; dibenamine hydrochloride, 50 mg. kg.; strychnine hydrochloride, 6.0 mg./kg.; picrotoxin (*B.P.*), 2.5 mg./kg.; insulin, 80 units/kg.; leptazol, 50 mg./kg.

The procedure for the extraction and estimation of glycogen and the separation of the brain into the different regions has been described elsewhere (Chance and Yaxley, 1950).

RESULTS

Normal values for glycogen in the different regions of the brain are given in mg./100 g. in Table I.

TABLE I

GLYCOGEN CONTENT OF NORMAL MOUSE BRAIN

Part of brain	Glycogen mg./100 g.	S.D.	No. of samples
Cortex	7.5	±0.38	22
Mid-brain	27.5	±0.49	22
Medulla	17.4	±0.56	22
Spinal cord	23.5	±0.48	4
Cerebellum: Central lobe	27.2	±0.77	4
Cerebellum: Lateral lobes	24.1	±1.40	4

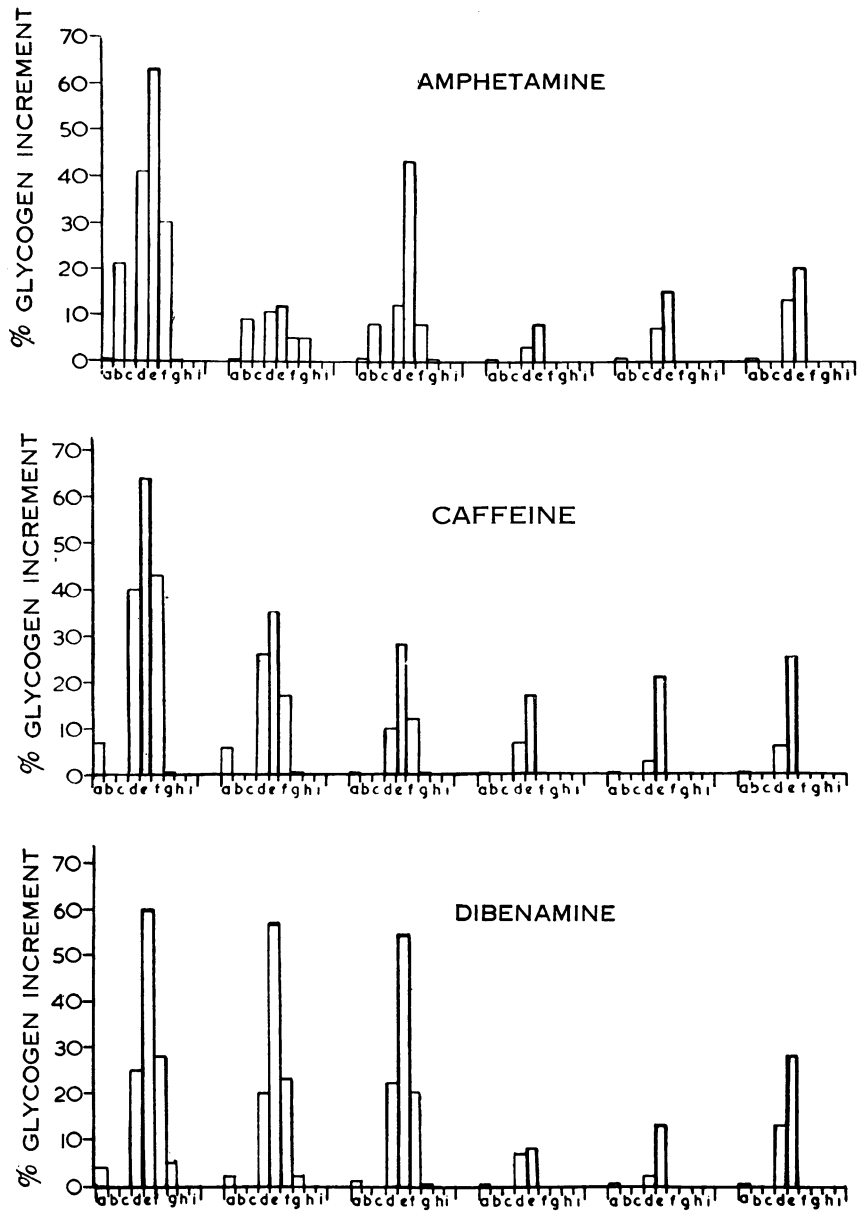
The results have been depicted in histograms (pp. 3–5) in which glycogen increments are expressed as percentages of the normal values for each part of the brain and are drawn against time for each drug. Each block in the histogram is the mean of three separate determinations. It has already been shown (Chance and Yaxley, 1950) that the variance of the increment is very low, and this has been found consistently throughout the present investigations. The mean standard deviation of the percentage increment, calculated from eighty sets of three determinations selected at random, was 1.65. The mean standard deviation, therefore, for the difference between two sets is $1.65\sqrt{2}$, which equals 2.33. Thus for a P value of 0.05 the difference between two percentage increments, each the result of three estimations, will be significant if they differ by more than 4.66 per cent ($2 \times 1.65\sqrt{2}$). This statement holds true for all levels of response, since the variance is independent of the response. All comparisons of the values for the individual blocks in the histograms are made on the assumption, therefore, that a difference has been recorded only if the means differ by 5 per cent or more. This criterion is slightly more rigorous than the statistical data warrant, but serves to emphasize the accuracy of the method.

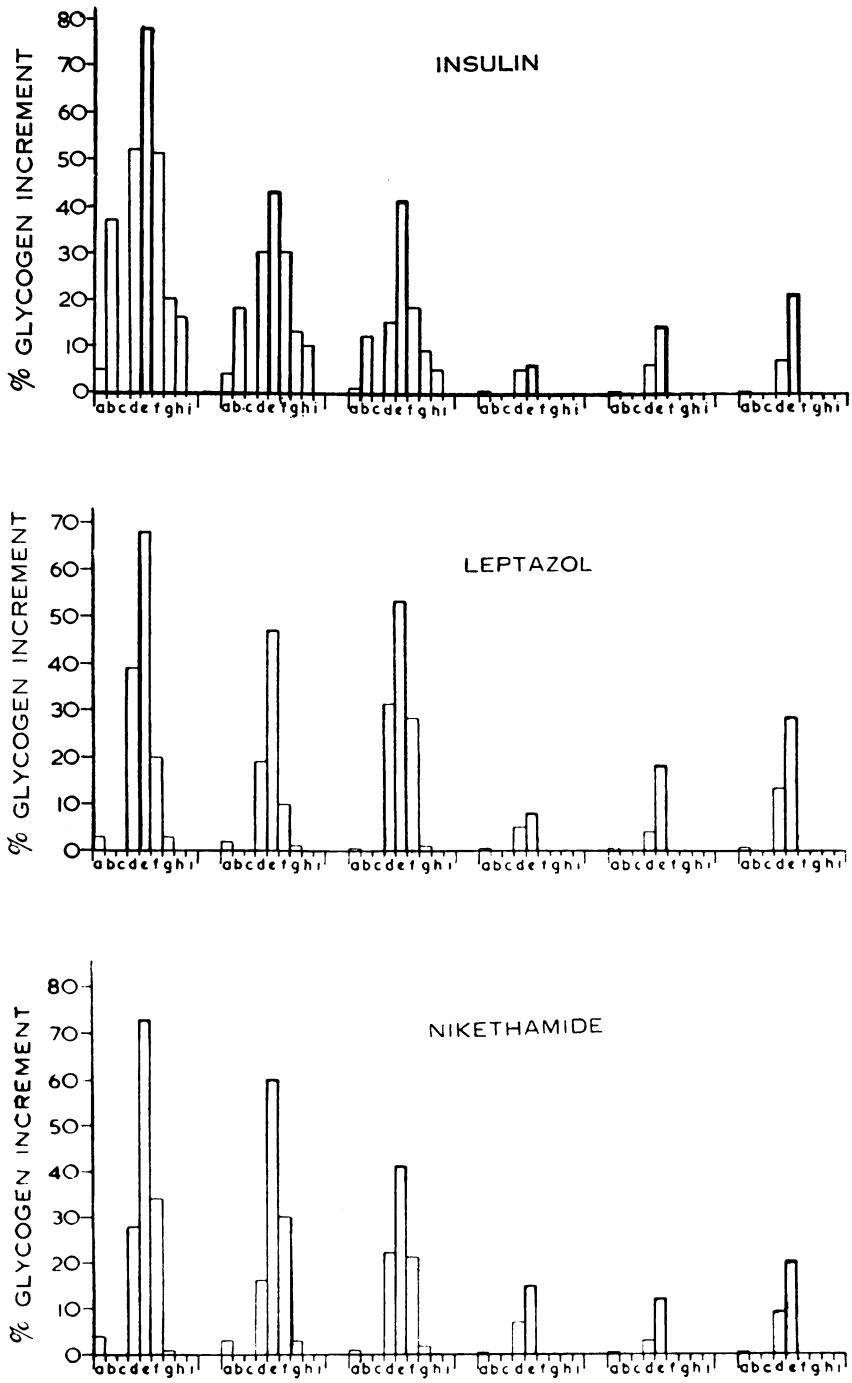
If these histograms are compared taking into account the rate of the rise in any one part of the brain (comparison eight seconds after start of convulsion), the peak rise and the relative rate of loss following the peak rise, it will be seen that each drug presents a distinctive pattern.

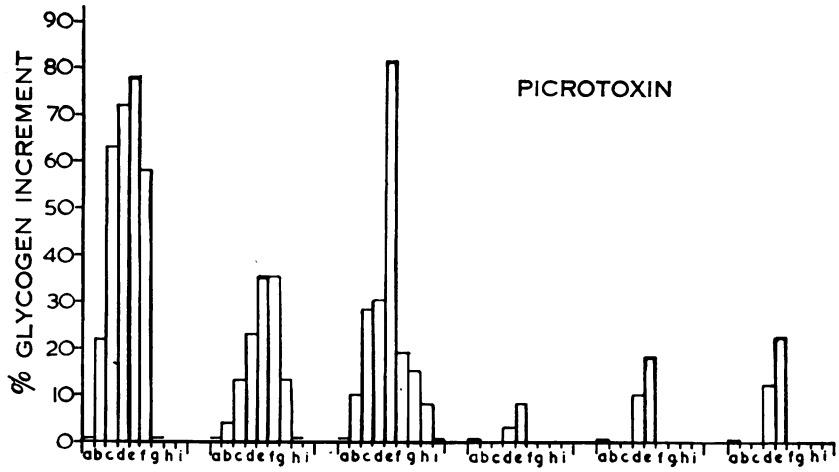
All the drugs except dibenamine and picrotoxin produced larger increments in the cortex than in other parts of the brain.

All the drugs produced smaller increments in the cerebellum and the spinal cord than in the other three parts of the central nervous system. Whereas all the other drugs produced a mid-brain increment greater than 34 per cent, amphetamine produced an increment in this region of only 13 per cent, which was about the same as that produced in the spinal cord and cerebellum. This is in marked contrast to the cortical and medullary increments, which were at least three times as large as that in the mid-brain.

HISTOGRAMS.—Ordinates: percentage glycogen increment. Abscissae: parts of the brain are arranged *from left to right* as follows: cortex, mid-brain, medulla, spinal cord, central lobes, and lateral lobe of the cerebellum. Time intervals are indicated along the abscissae by the letters *a* to *i*, thus: (*a*) preconvulsion; (*b*) start of convulsion; (*c*) 5 sec. after *b* for picrotoxin only; (*d*) during convulsion; (*e*) end of convulsion; (*f*) 20 min., (*g*) 40 min., (*h*) 60 min., and (*i*) 90 min. after convulsion.




INSULIN
LEPTAZOL
NIKETHAMIDE
% GLYCOGEN INCREMENT
0
10
20
30
40
50
60
70
80
abcdefghi


% GLYCOGEN INCREMENT
0
10
20
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40
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70
80
90
PICROTOXIN
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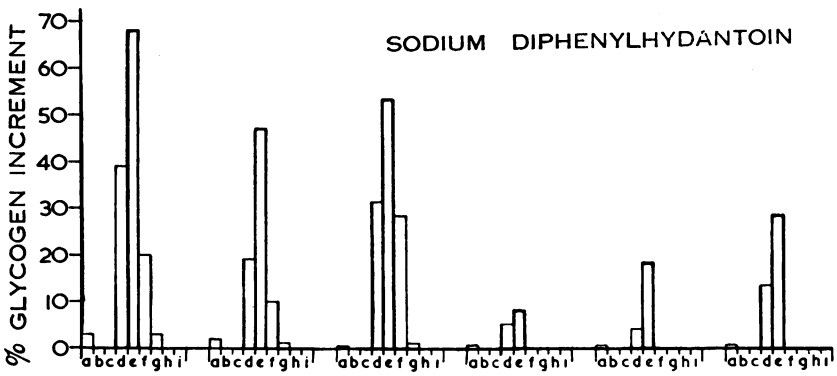
% GLYCOGEN INCREMENT
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50
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70
SODIUM DIPHENYLHYDANTOIN
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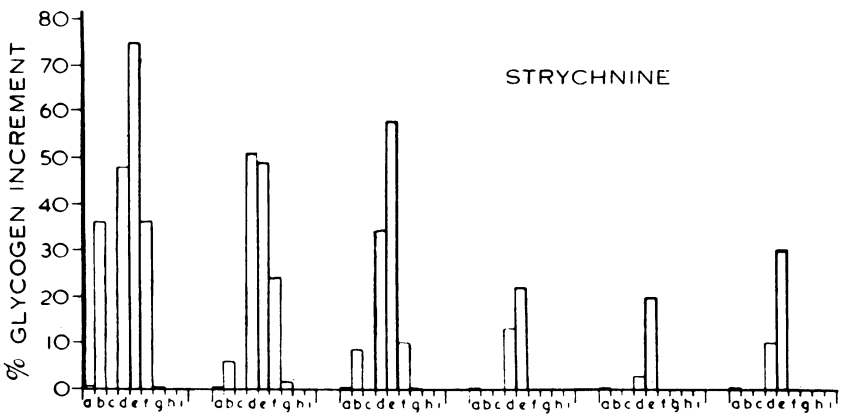
% GLYCOGEN INCREMENT
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70
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STRYCHNINE
abcdefghi abcdefghi abcdefghi abcdefghi abcdefghi abcdefghi

The time elapsing between the injection of the drug and the onset of the convulsion was for most of the drugs no longer than thirty seconds, and the convulsion itself did not last longer than a minute. Picrotoxin was the only exception. The induction period for this drug lasted from two to four minutes and convulsions occurred sporadically over a period of a further five minutes. These facts must be remembered when assessing the relative rates of increment induced by the different drugs in a particular part of the brain. It will be noted that the apparently sudden increment in the medulla towards the end of the picrotoxin convulsions does not represent an exceptionally rapid rise whether this is estimated on the basis of the total time occupied by the convulsions or in relation to the time span itself.

If a comparison of the increments in the separate parts of the brain due to the different drugs is made (eight seconds after the start of the convulsion), it will be seen that four drugs (insulin, picrotoxin, amphetamine, and caffeine) produced a more rapid rise in the cortex than in the rest of the brain.

Picrotoxin stands out in contrast to all the others, in that it produced a final increment of glycogen in the medulla equal to, if not in excess of, the rise in the cortex, both these parts showing an 80 per cent increment. This cortical increment was only equalled during convulsions produced by insulin and by leptazol. Picrotoxin and amphetamine and to a lesser extent leptazol and sodium diphenyl-hydantoin produced larger increments in the medulla than they did in the mid-brain. Dibenamine was unique in that the increments in all the three higher regions of the brain were identical.

The rate of recovery was the same in the three higher parts of the brain after convulsions produced by caffeine, nikethamide, dibenamine, sodium diphenyl-hydantoin, and strychnine. The rate of recovery in the mid-brain after amphetamine convulsions was very slow, and this was probably due to the small extent of the rise which occurred in this part. Recovery was also noticeably slower in the later stages both in the mid-brain and medulla after picrotoxin convulsions and in the three higher regions after insulin convulsions.

Discussion

The evidence we have presented elsewhere (Chance and Yaxley, 1950), showing the intimate relationship of the glycogen increments with convulsive activity, suggested the present study, which describes the patterns of nervous activity characteristic of each drug. While at present there is not sufficient evidence to draw any conclusions about the relationship between convulsive activity in the central nervous system and the nervous activity of the cells during the convulsion, it is probable that there is some direct relation between these two phenomena. The method has demonstrated that characteristic patterns of glycogen increment are produced by a number of central nervous convulsions, and it is reasonable to infer that this reflects a patterned interference with the biochemical processes involved in the early stages of carbohydrate utilization. It is, however, not possible to infer that the differential increments are a result of the direct action of the drug on the particular part of the brain in which the increment has occurred. Before this is known it will be necessary to assess how far the activity engendered in one part of the brain modifies the increment in another.

The increments are expressed as a percentage of the normal values of the particular part of the brain under examination. The absolute values for each part differ considerably, and this (on the assumption that the glycogen is intracellular) must depend upon the number of neurones present in a given sample, i.e., on the proportion of grey to white matter and glial tissue. Since the same percentage increment can be produced in all parts of the brain by electrical convulsions (Chance and Yaxley, 1950) it is logical to assume that there are no major inherent differences limiting the extent of glycogen increment in any one part. If this is so then we have shown that the amount of activity in the spinal cord and cerebellum during clonic convulsions produced by these drugs is much less than the activity in the higher brain centres.

Owing to the restricted use of picrotoxin as an antidote to barbiturate poisoning, our knowledge of this drug, unlike that of the majority of central nervous stimulants, is derived from the use of doses which in the absence of the antidote would induce convulsions. It is interesting to compare, therefore, the action of this drug as revealed by the " convulsive " pattern with what we know about it. Picrotoxin produces, as might be expected, its maximum increment in the medulla, which is consistent with our knowledge that it is a potent stimulant of the respiratory centre. But it is also equally effective in stimulating the cortex; in fact the stimulation appears to start in the cortex, and to last longer there than in the medulla. It is interesting to note that, as early as 1875, Crichton Browne suggested that picrotoxin was " more than a medullary stimulant " and had some action on the cerebral cortex. It may be the reason for its peculiar effectiveness as an antidote to barbiturates, and in particular may be the explanation why the barbiturates can be " titrated " against it and *vice versa*. On the basis of the similarity between the glycogen increment curve of picrotoxin and leptazol we should expect that the latter substance would be the next most effective antidote, and indeed it is. Strychnine, which has some slight antidotal properties against lethal doses of barbiturates, showed some similarity to the aforementioned drugs, in that the increment in the medulla is of the same order as that produced by leptazol. It differed, however, from them in that the increment in the mid-brain comes very close to that in the medulla. It is interesting to note that this substance, regarded as a spinal stimulant, produced the largest spinal increment (23 per cent) of all the drugs examined.

SUMMARY

The very low variance associated with the estimation of glycogen in mouse brain during procedures which bring about changes in amount of this metabolite makes it possible to estimate the relative changes in different parts of the brain with exceptional accuracy. The accumulation of glycogen in the brain of the mouse, which takes place during a convulsion, has been estimated in different parts of the central nervous system during seizures produced by convulsant drugs. The resulting patterns are interpreted as indicating the *distribution* of nervous activity in the brain arising from the stimulation of the drug.

REFERENCES

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