19. Which ONE of the following enzymes is **NOT** activated in the signalling sequence following glucagon binding to its receptor on a liver cell?

a) glucose-6-phosphatase

b) phosphorylase kinase

c) adenylate cyclase

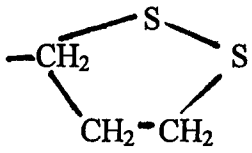
d) protein kinase A

20. Which ONE of the following is **NOT** carried out by the action of dihydrolipoamide dehydrogenase in the PDH complex of mitochondria?

a) The reduction of FAD (flavin adenine dinucleotide)

b) The oxidation of NADH to NAD^+, a reaction required for the reduction of FAD noted in a) above.

c) The oxidation of dihydrolipoamide to the structure:

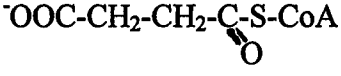


d) The regeneration of the enzyme dihydrolipoamide acetyltransferase with its lipoamide coenzyme

21. Which ONE of the following, concerning the Krebs cycle, is **INCORRECT**?

a) The only tricarboylic acids in the cycle are produced by the actions of citrate synthase and aconitase.

b) The least favourable oxidoreductase reaction in the cycle produces the compound:



c) The only substrate level phosphorylation reaction in the cycle is catalyzed by succinyl CoA synthetase

d) One turn of the cycle, utilizing one molecule of acetyl CoA, will require 2 molecules of water and produce 2 molecules of CO_2

22. Which ONE of the following, concerning electron transport, is INCORRECT?

a) The addition of reduced flavin mononucleotide ($FMNH_2$) to mitochondria poisoned with amytal will not allow electron transport to resume.

b) Two molecules of reduced cytochrome c should have a P/O ratio = 1.0 (in classic terms) as shown by the equation:
2 cytochrome c (Fe^{2+}) + ADP + Pi + ½ O_2 → 2 cytochrome c (Fe^{3+}) + ATP + H_2O

c) Mitchell's proton motive force is generated by the pumping of protons out of the matrix, across the inner mitochondrial membrane by the mitochondrial complexes: NADH dehydrogenase, ubiquinone/cytochrome c oxidoreductase and cytochrome c oxidase (Complexes I, III, IV).

d) Chemiosmotic theory relies on the establishment of a membrane proton gradient and this gradient can be diminished by damaging the inner mitochondrial membrane or by compounds, such as 2,4-dinitrophenol, that release protons into the mitochondrial matrix.

23. Which ONE of the following concerning gluconeogenesis is INCORRECT?

a) While rising concentrations of AMP increase rates of glycolysis, rising AMP concentrations will decrease the rate of gluconeogenesis.

b) The principal regulation points in the gluconeogenesis pathway are found at fructose-2,6-bisphosphatase and PEP carboxykinase.

c) Starting with 2 molecules of pyruvate, gluconeogenesis requires 4 molecules of ATP, 2 molecules of GTP and 2 molecules of NADH to allow the generation of 1 molecule of α-D-glucose.

d) The gluconeogenic pathway has two hydrolase enzymes that are not found in glycolysis.

24. Which ONE of the following is INCORRECT concerning fatty acid β-oxidation?

a) The enzyme enoyl CoA hydratase produces a new chiral C centre in its product.

b) Acyl CoA dehydrogenase produces a reduced coenzyme that has a classic mitochondrial P/O ratio = 2.

c) Thiolase produces an acyl CoA product that is 2 carbons shorter in length than its acyl CoA substrate.

d) Thiolase catalyzes a hydrolytic cleavage of ketoacyl CoA substrates.

25. In untreated Type I (Juvenile) Diabetes which ONE of the following is INCORRECT?

a) Triacylglycerol hydrolysis in fat cells is increased because of epinephrine stimulation, rising levels of cAMP and protein kinase A activation.

b) The actions of glucagon and epinephrine result in signalling sequences that accelerate the loss of glycogen reserves and the reduction of fat tissue reserves in the body.

c) There is increased activity of the carnitine shuttle system that involves the conversion of acylcarnitine to carnitine by the action of the translocase in the inner mitochondrial membrane.

d) The generation of ketone bodies by liver is so increased that a condition known as ketosis is produced, marked by high levels of ketone bodies in the blood, tissue dehydration, polyuria, loss of potassium ions from cells, kidney failure and coma.

SHORT ANSWER QUESTIONS
Total Value: 20 marks. Answer all the questions and place your answers in the exam books provided

26. **(4 marks)**
a) For the Case Study Nat, outline the signalling steps that follow the binding of TXA_2 to its receptor on the platelet plasma membrane and ultimately result in platelet shape changes and release of platelet granule contents.

b) Explain how a diet rich in salmon can reduce platelet activation noted in a) above.

27. **(4 marks)**
a) What is meant by the term phospholipid asymmetry, applied to the plasma membrane of cells?

b) What experiment could you design to show this phospholipid asymmetry?

28. **(4 marks)**
a) Explain what is now considered the most important source of cholesterol found in patients who have high levels of serum cholesterol, indicate how these high levels of cholesterol can be controlled, and explain why some patients see a rise in their serum cholesterol levels if they eliminate this steroid from their diet.

b) What is the most important defect that causes elevated cholesterol levels in the blood?

CONTINUED...

29. **(4 marks)**
a) Calculate how much ATP is made when one molecule of pyruvate is placed within the matrix of a mitochondrion. Consider the complete breakdown of the pyruvate molecule in mitochondria by the action of the PDH complex, the Krebs cycle, electron transport and oxidative phosphorylation. Assume classic P/O ratios. Show your calculations.

b) For the mechanism of the enzyme glyceraldehyde-3-phosphate dehydrogenase, give the structure of the thiohemiacetal-enzyme intermediate and show how it is formed.

30. **(4 marks)**
Elizabeth Hughes was the young type I diabetic patient treated successfully by Fred Banting. When she first came to see Fred she was virtually a walking skeleton, but after she began her insulin treatment she put on weight very quickly.

Considering Elizabeth's liver, describe the dramatic biochemical events that happened after she started her insulin injections.

**SEE NEXT PAGES FOR ASSIGNMENT

Assignment (15 marks)
Please place your answers in a NEW lined exam booklet

Part 1: (7 marks)
The three figures taken from the Zhu et al. publication in the Journal of Neuroscience are given on p. 14 and 15). Using these results please answer the following questions using a separate examination booklet. You can use point form if you wish. **Please write legibly.**

1. Based on the results in Figure 1 (p.14), ischemia and reperfusion (without creatine) produce a considerable stroke volume. In Figure 3 (p.15) these results are supported by depressed ATP levels in this ischemic region. If the researchers had analyzed areas within the ischemic region over time, what results would they have found? (2 marks)

2. In Figure 2 (p.14), explain how the researchers were able to distinguish between active and inactive caspase 3. If they found in cases of ischemia (with no creatine) a distinctive band near the bottom of the gel, what would this signify? (2 marks)

3. In Figure 3 (p.15), why did the researchers assay for ATP and creatine after 30 min of MCA occlusion, instead of 2 hours of MCA occlusion and 24 hr of reperfusion? (1 mark)

4. The researchers found that creatine had to be administered for a month to show neuorprotection. Propose a mechanism that would explain this observation. (2 marks)

Part 2: (8 marks)
The three figures given to you before are now shown on pages 16 and 17. As well the experimental details that you were given are also provided on page 15.

5. Noting the results in Figure1 (p.16), what advantage is there in measuring phosphocreatine instead of creatine in the contralateral and ipsilateral tissue samples? (1 mark)

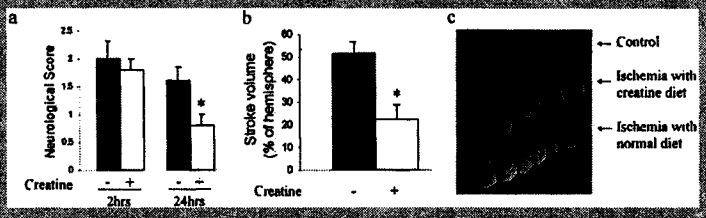
6. In Figure 1 (p.16) it appears that GB438 treatment does little to preserve ATP and phosphocreatine levels in this ischemic tissue. Why do you think this is so? (1 mark)

7. Using the results from Figure 2 (p.17), is there evidence that GB438 has a role in preventing ischemia-mediated cell death? Explain your answer based on apoptotic mechanisms and possible effects of TNFα (2 marks)

8. As seen in Figure 2 (p.17), with ischemia and no creatine or GB438, increased levels of active caspase 8 are accompanied by increased levels of Bid fragments. Yet with ischemia and creatine, there is active caspase 8 but little Bid fragmentation. Propose what might account for this observation (2 marks).

9. Based on the results in Figures 1 and 2 propose a mechanism whereby creatine administration may regulate cytochrome c levels in the cytosolic fraction. (2 marks)

Figure 1. Protection from MCA occlusion-mediated injury in creatine-treated mice.



Zhu, S. et al. J. Neurosci. 2004;24:5909-5912

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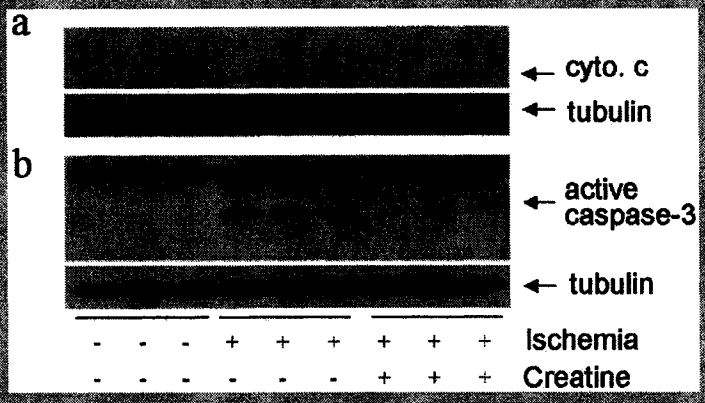
Figure 2. Caspase-3 activation and cytochrome c release in ischemic brain territories after 2 hr of ischemia and 24 hr of reperfusion.



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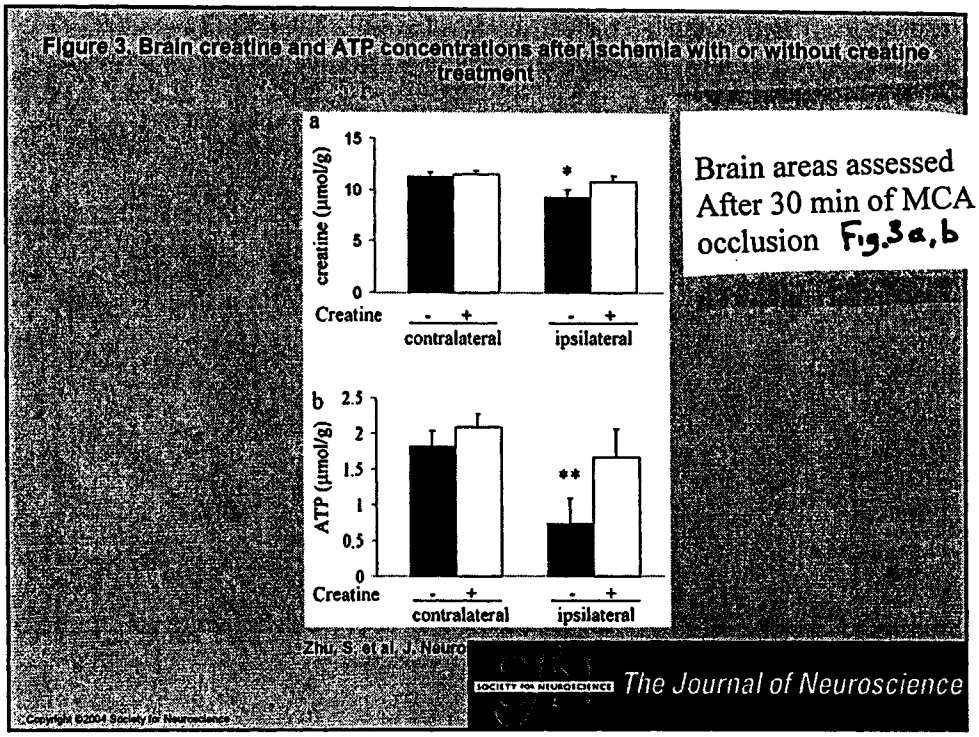

Brain areas assessed
After 30 min of MCA
occlusion Fig. 3a, b

PART TWO

Hypothesis: Tumor necrosis factor α (TNFα) produced in dying cells in ischemic tissue is responsible for part or all of the reduction in ATP levels seen in ischemic brain, and dietary creatine decreases the effects of TNFα.
Methods: The same mouse focal brain ischemia model is used as described in the Journal of Neuroscience paper. Mice are divided into four groups and each group has 4 animals:
Group 1 receives no therapeutic agents prior to ischemia.
Group 2 is given intravenous GB438, a specific blocker for the TNFα receptor, starting the day before ischemia.
Group 3 receives dietary creatine (2% in diet) four weeks before ischemia
Group 4 receives dietary creatine (2% in diet) four weeks before ischemia and intravenous GB438 starting the day before ischemia.

As described in the J. Neuroscience paper, brain tissue is taken from the ipsilateral side (ischemic area) and contralateral side (matching non-ischemic area) of each brain. The tissue is homogenized and analyzed for ATP and creatine (by HPLC), or cytochrome c and caspase 3 (by Western blots). These components of brain tissue are measured using the same methods described for the J. of Neuroscience paper.

Results:

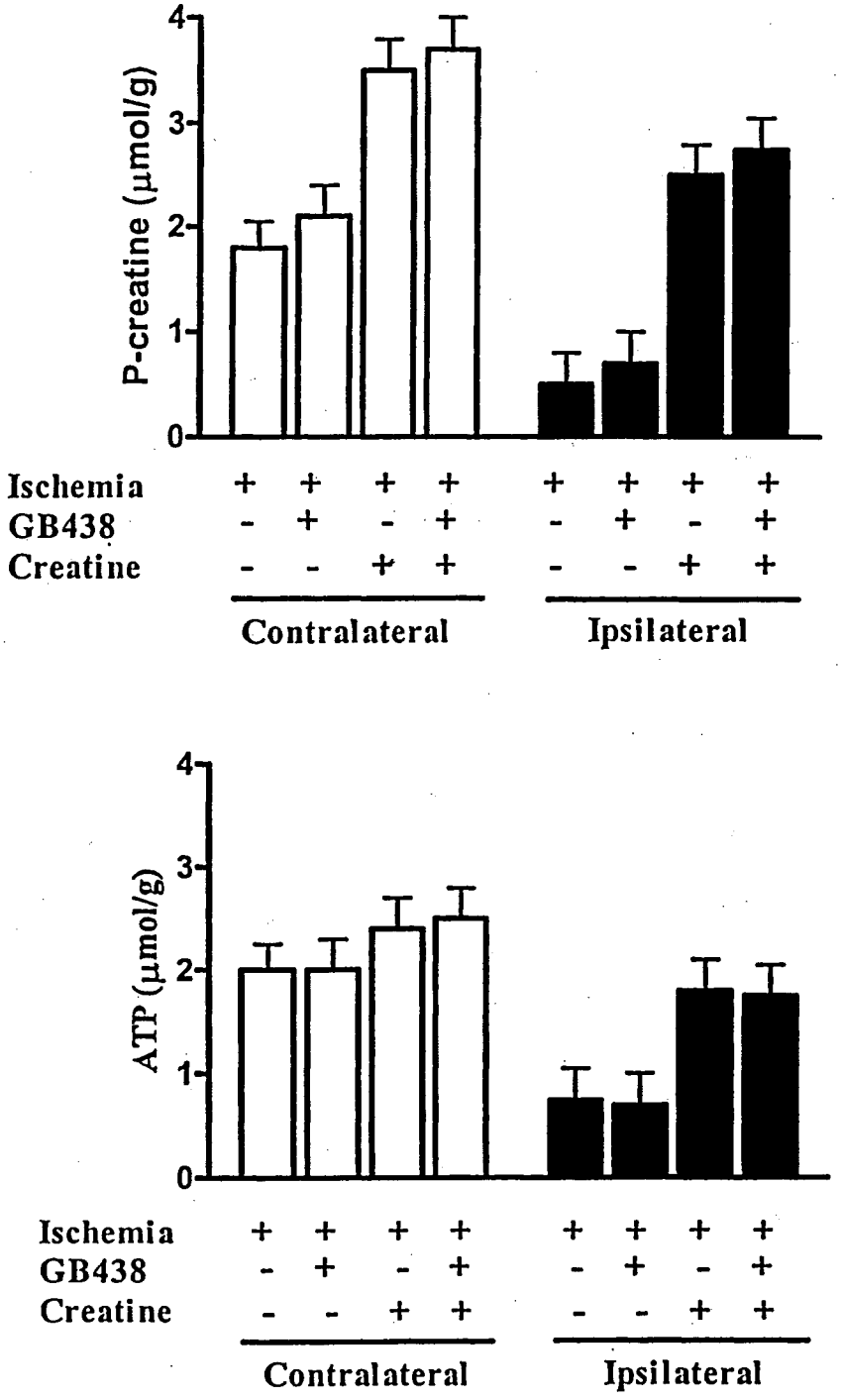

Figure 1 ATP and phosphocreatine levels in the ipsilateral and contralateral sides of brains taken from each Group after 30 min of MCA occlusion. The values are means ± standard deviation of values found for 4 different animals. Each value found for the ipsilateral side was significantly different from the result for the matching contralateral side.

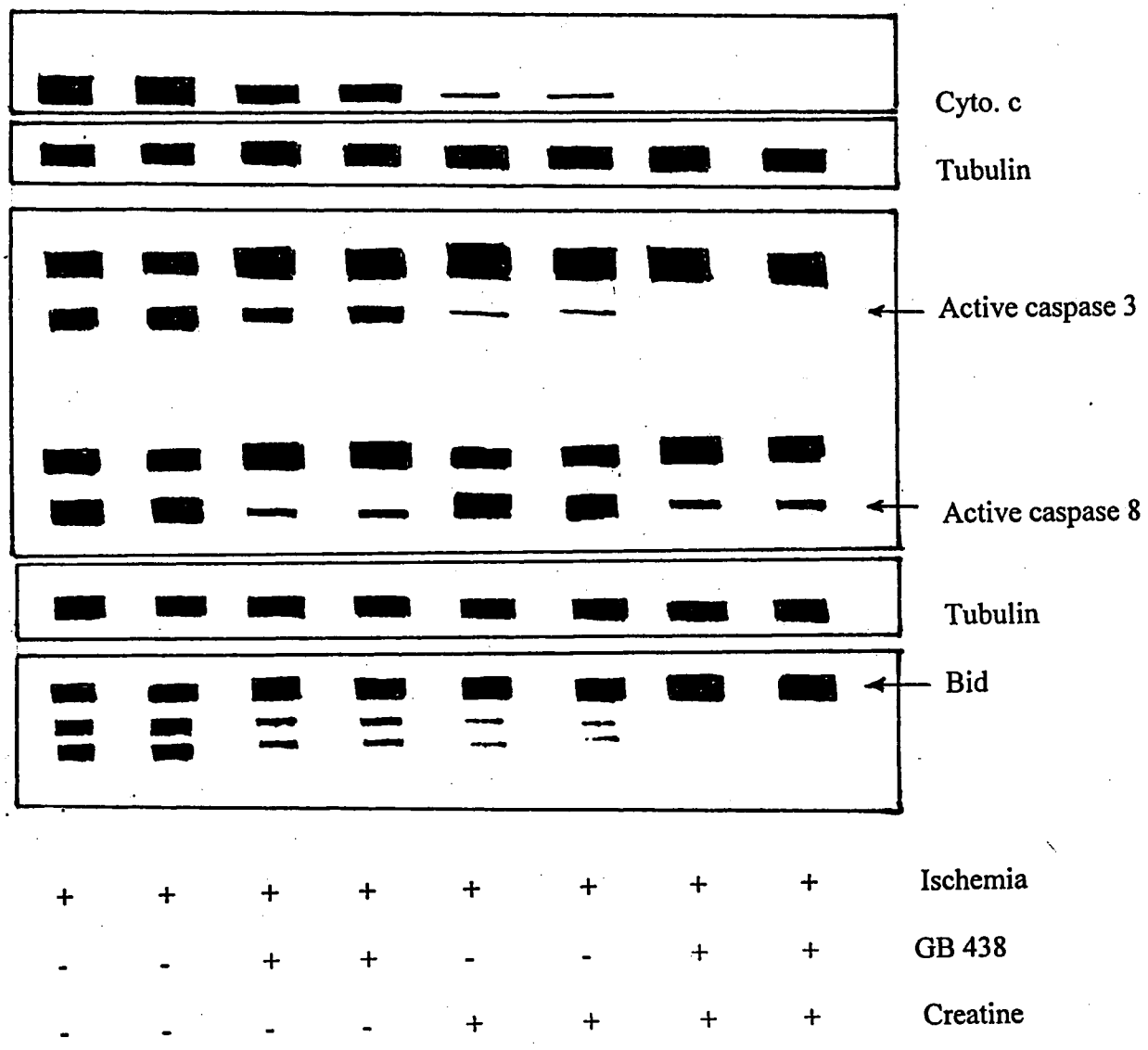

Figure 2 Western blot of brain extracts. This blot shows the bands of proteins isolated from extracts of ischemic brain tissue (ipsilateral side) after 2 hours of ischemia and 24 hours of reperfusion. Cytochrome c release was determined using cytosolic extracts, and other proteins were assessed using brain homogenates (lysates). Western blots were carried out using specific antibodies to cytochrome c, caspase 3, caspase 8, Bid and tubulin. Each lane represents an individual mouse