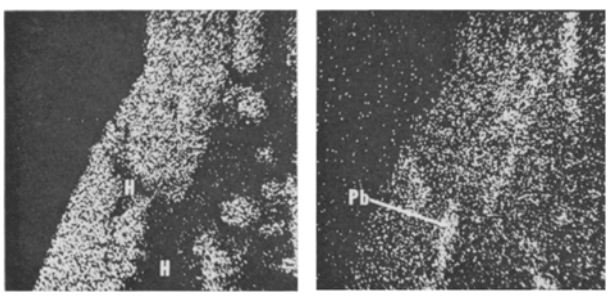
uniform calcium and phosphorus emissions) and 'hypomineralization' (a deficiency of calcium and phosphorus emissions) were noted. In almost all cases, the lead was deposited either in or at the periphery of the 'hypomineralized' zones. The same pattern of deposition was also noted in the root dentine, especially in areas adjacent to the pulp. This localization effect was found in teeth of asymptomatic children and in those diagnosed as having suffered from lead poisoning. No lead was detected in surface enamel. This was a surprising finding in view of the report of BRUDEVOLD et al.[3] that a very high percentage of lead occured in the most superficial layers of this tissue.




X-ray scanning images for phosphorus (left) and lead (right). The region shown in this figure (200 μm × 200 μm) is close to the cemento-enamel junction. Areas of dense lead localization (Pb) can be seen to approximate to 'hypomineralised' zones (H) of the phosphorus scan. The calicum S-ray scan (not shown here) was identical to the phosphorus image.

The presence of a considerable quantity of lead in the body of the dentine and in the enamel suggests that lead is incorporated into the tooth structure during matrix formation and mineralization. Furthermore, the appearance of lead in root dentine indicates that it is also taken up by the tooth during root formation and the deposition of secondary dentine. Thus, chemical analysis of the tissues formed before and after tooth eruption could be used to provide a history of lead ingestion during different phases of the life cycle of the tooth[4].

*Résumé.* Douze dents de lait d'enfants citadins, dont deux ayant une intoxication saturnine reconnue, ont été examinées par un microanalyseur à sonde électronique. On a retrouvé du plomb dans toutes les dents, avant tout à la périférie des zones hypominéralisées; mais pas à la surface de l'émail. Les résultats suggèrent que le plomb est incorporé dans la dent pendant la formation de la matrice et sa minéralisation, ainsi que durant la formation de la racine et du dépot de dentine secondaire.

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*24 September 1971.*

[4] Acknowlegment. This work was supported by USPHS grant No. DE-02623.
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## Relationship Between Hypothermia and some Chlorpromazine Induced Metabolic Changes in Mouse Brain

The effects of chlorpromazine (CPZ) on the rapid conversion of glucose into amino acids and its relationship to chlorpromazine induced hypothermia have been investigated.

*Methods.* Female SAS/ICI albino mice, 25–40 g, were given 0.9% saline, 5 ml/kg or CPZ 20 mg/kg i.p. 30 min after this they were given 5 μCi (U-$^{14}$C)-D-Glucose i.p. The animals were killed 30 min afterwards and the cerebral hemispheres rapidly frozen in liquid nitrogen, homogenized in 3 ml of ice-cold 10% trichloracetic acid (TCA) and then chemically fractionated and the radioactivity of the fractions determined by liquid scintillation counting[1].

For the estimation of individual amino acids brain samples were extracted with a total of 9 ml 80% ethanol and 5 ml distilled water. This extract was passed through a Zeo-Karb 225 resin column in the $H^+$ form to separate the amino acid containing fraction. The eluate was analyzed by quantitative paper chromatography[2]. The percentage recovery of radioactivity was 68.5 ± 13.3% ($N = 13$) and amino acid levels were individually corrected to 100%. Glutamine concentration and the combined concentrations of glutamic acid, γ-aminobutyric acid and glutathione (GGG) were estimated colorimetrically[3].

The body temperature was measured by a rectal thermistor probe. Overall, the mean rectal temperatures in which body temperature was maintained (by placing the mice in a 38–40 °C incubator) were: control 37.65 °C ($N = 20$); CPZ 37.80 °C ($N = 19$). For animals with uncontrolled body temperatures these were: control 37.40 °C ($N = 26$); CPZ 29.20 °C ($N = 24$).

The results were examined by *t*-test or an analysis of variance using the multiple comparison technique[4].

*Results.* The effect of CPZ on the uptake of radioactivity is shown in Table I. There is no significant change in total uptake of radioactivity but the percentage incorporation of radioactivity into the TCA fraction is markedly increased by CPZ during hypothermia and is reduced but not abolished when body temperature is maintained. The relationship between radioactivity in the TCA fraction and temperature of animals treated with CPZ is shown in the Figure. When the body temperature was allowed to fall, no significant change in individual amino acids was produced (Table II).

The percentage radioactivity of the amino acids of the total TCA fraction was: control 70.9% (S.D. = 16.1; $N = 7$); CPZ 67.9% (S.D. = 11.1; $N = 6$); significance of difference: $P > 0.05$; $t = 0.4$.

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Table I. The effect of CPZ on the uptake of radioactivity into mouse brain following administration of (U-$^{14}$C)-D-glucose

| | CPZ | Control | Difference ±95% confidence limits | P |
|---|---|---|---|---|
| | Hypothermic | | | |
| Total Uptake counts $min^{-1}$ $mg^{-1}$ | 463 | 563 | 100±193 | N.S. |
| Incorporation into TCA fraction (%) | 97.12 | 94.09 | 3.02±0.98 | <0.001 |
| | Normothermic | | | |
| Total Uptake counts $min^{-1}$ $mg^{-1}$ | 737 | 791 | 54±38 | N.S. |
| Incorporation into TCA fraction (%) | 95.84 | 95.05 | 0.79±0.41 | <0.05 |

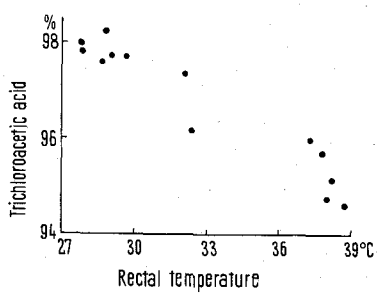
N.S. = non-significant at $P$ 0.05.

Table II. The effect of CPZ on the absolute levels of amino acids in brains from mice whose body temperature was not controlled

| Amino acid ($\mu$M $g^{-1}$ wet weight ± S.D.) | CPZ ($N$ = 6) | Control ($N$ = 7) |
|---|---|---|
| GABA | 3.1±0.8 | 2.9±0.7 |
| Alanine | 1.2±0.3 | 1.1±0.6 |
| Glutamine | 5.7±3.8 | 3.0±1.8 |
| Glycine and serine | 2.9±0.8 | 2.7±0.5 |
| Glutamic acid | 13.1±2.5 | 14.4±3.9 |
| Aspartic acid | 2.9±1.1 | 3.2±1.0 |

Table III. The effect of CPZ and body temperature on the concentration of glutamine and the combined concentrations of glutamic acid, GABA and glutathione (GGG) expressed as $\mu M$ $g^{-1}$±S.D.

| | CPZ | Control |
|---|---|---|
| | Hypothermic | |
| Glutamine | 8.2±2.5 | 5.8±1.6 |
| GGG | 24.6±1.8 | 27.8±4.5 |
| | Normothermic | |
| Glutamine | 8.1±2.8 | 12.9±4.6 |
| GGG | 28.9±4.7 | 23.7±3.6 |




Relationship between incorporation of isotope from (U-$^{14}$C)-D-Glucose into the TCA fraction of mouse brain and maintained rectal temperature in mice treated with CPZ 20 mg/kg i.p.

The effect of CPZ and body temperature on the concentration of glutamine and the combined concentrations of glutamic acid, GABA and glutathione (GGG) is shown in Table III. Analysis of variance suggests that none of the differences apparent on inspection are significant at $P$ 0.05.

*Discussion.* When the temperature of CPZ treated animals is allowed to fall the fraction of radioactivity incorporated from glucose into the TCA fraction is raised and this effect is decreased by maintenance of body temperature. There was no effect in drug treated hypothermic animals on the incorporation of glucose carbon into brain amino acids. This conflicts with the work of BACHELARD and LINDSAY[5] who found that CPZ lowered the relative specific activity of amino and keto acids in the brains of hypothermic rats although BACHELARD, GAITONDE and VRBA[6] found no change in the percentage of total $^{14}$C in the free amino acid fraction of brain from CPZ treated normothermic rats.

The present work shows no significant changes in the absolute levels of some selected amino acids after CPZ hypothermia. Although a number of contradictory reports concerning small effects of CPZ on the levels of different amino acids exist there is little reason to revise the opinion of TALLAN[7] that CPZ has but slight effect on the overall pattern of brain amino acids. A CPZ induced rise in brain glutamine has been reported[8] although BACHELARD and LINDSAY[5] found a decreased $^{14}$C incorporation into this compound. Hypothermia alone produces increased brain glutamine levels[9]. Furthermore, the inhibition of brain glutamic acid dehydrogenase by low concentrations of CPZ would favour an increase of brain glutamine[10]. The results indicate that CPZ increases brain glutamine compared to the normothermic control whether body temperature is normal or reduced.

The CPZ inhibition of cerebral protein synthesis is temperature dependent[1] and the present work shows that

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the increase in $^{14}C$ in the TCA fraction is not due to its incorporation into amino acids and thus much of this increased radioactivity may occur as glucose or glycolytic metabolites. GEY, RUTISHAUSER and PLETSCHER[11] have described a rise in brain glucose in CPZ hypothermia in rats, which they attributed to suppression of glycolysis, but prevention of hypothermia did not abolish changes in carbohydrate metabolism produced by CPZ[12].

*Zusammenfassung.* Es wird gezeigt, dass Chlorpromazin keine Veränderung der Konzentration von Aminosäuren erzeugt, wohl aber die Inkorporation des Isotopen $^{14}C$-Glukose im säurelöslichen Anteil des Mäusegehirns temperaturabhängig vermehrt.

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[13] Governors' Research Scholar, Guy's Hospital Medical School.

## The Relationship of the Renal Vasodilator Action of Bradykinin to the Release of a Prostaglandin E-Like Substance

Kinins have variable effects on blood vessels; viz., in vitro constriction usually occurs, whereas in vivo most vascular elements dilate, although venoconstriction is the rule[1]. The diverse vascular effects of kinins are evident in the actions of bradykinin on the fetal circulation. Bradykinin constricts the umbilical vessels and the ductus arteriosus, and dilates the pulmonary vasculature which effects have occasioned the proposal that a kinin mediates neonatal circulatory changes[2]. If some of the effects of kinins on blood vessels can be shown to be dependent on release of a mediator, then their diverse vascular actions may be made comprehensible. Postaglandins of the E (PGE) and A (PGA) series have been suggested to be local mediators of stimuli evoking vasodilation[3]. We undertook the present study in an attempt to relate the renal vasodilator action of bradykinin to the release of prostaglandins. The renal circulation was selected since renal blood flow (RBF) is highly sensitive to prostaglandins and kinins[4,5] and the predominent renal prostaglandin, $PGE_2$[6,7] is released by vasoactive hormones[8].

*Methods.* Male mongrel dogs (22–31 kg) were anesthetized with morphine sulfate (2 mg/kg, s. c.) and chloralose (100 mg/kg, i.v.). The abdominal cavity was opened by a transverse incision and a renal artery isolated. Two Hewlett-Packard direct writers recorded: a) mean aortic blood pressure measured by a Statham transducer; b) RBF measured by a Statham electromagnetic flowmeter and c) changes in length of assay organs detected by Harvard isotonic transducers. We have reported the adaptation of the blood-bathed organ technique of VANE[9] for continous assay of prostaglandins in renal venous effluent[10] (Figure). In brief, 3 assays organs: rat stomach strip, rat colon and chick rectum, were superfused (streaming of fluid over assay organs) in series by renal venous blood withdrawn by a pump at 15 ml/min and returned to the dog via the left jugular vein. The assay organs in vitro were superfused with Krebs solution in order to estimate concentrations of PGE- and PGF-like substances in purified extracts of renal venous blood. Renal venous blood (100 ml) was collected in ethanol before and during infusion of bradykinin into the renal artery. Heparin (1500 IU/kg) was given i.v. just prior to superfusing the assay tissues. Dextran was infused i.v. at the same rate as renal venous blood was removed. The ethanolic-blood mixture was filtered, evaporated and the acidic lipids separated from the neutral lipids as previously described[11]. The acidic lipids were further purified by thin-layer chromatography on silica gel layers, 0.5 mm thick, using the solvent system: chloroform: methanol: acetic acid (18:1:1 by vol.). Eluates from thin-layer chromatographic zones were reconstituted in Krebs solution to make a final dilution of 0.5 ml; 0.1 ml volumes of the latter were assayed in vitro for prostaglandins. Since the minimum amount of $PGE_2$ standard which produced a measurable response of the assay organs varied between 0.1 and 0.3 ng, the threshold of sensitivity of this assay system for PGE-like substances expressed as $PGE_2$ equivalents was always 0.015 ng/ml blood or less. Thus, the sensitivity of this assay for $PGE_2$ is well below the threshold value of $PGE_2$ of 0.1 ng/ml blood which increases RBF[4]. The concentration of PGE- and PGF-like substances in the eluate was determined by bracket assay (Figure). The medians of the coefficients of variation of the assay system were 10.9 and 12.9% respectively for duplicate and replicate determinations of the concentration of prostaglandins in the eluates. Concentrations of prostaglandins were not corrected for losses (average 38%) incurred on extraction and purification.

*Results.* Bradykinin, given into the renal artery by infusion (20 to 100 ng/kg/min) or single shot 40 ng/kg, increased RBF by 16 to 110% of control (mean increase 58%). Aortic blood pressure was unchanged from the mean control value of 96 mm Hg. In all experiments, contraction of the assay organs bathed by renal venous blood occurred in response to close-arterial administration of bradykinin (Figure). Bradykinin presumably released a

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