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## Effect of Urea on Enzyme Activity of the Choroid Plexus. (25099)

ROBERT G. FISHER, JOHN H. COPENHAVER, JR., AND DAVID S. MALINE (Introduced by George H. Stueck, Jr.)
*Dartmouth College and Medical School, Hanover, N. H.*

Fremont-Smith and Forbes(1) demonstrated that urea when injected into peritoneum of the cat had a rather striking effect on reduction of intracranial pressure. They suggested its clinical application because of its lack of toxicity and its ready elimination by normal kidneys. Smythe, Smythe and Settlage(2) later found that 17% urea produced a profound and persistent drop in intracranial pressure. On this basis, Javid and Settlage(3) reported effective use of a 30% solution of urea in reducing the intracranial pressure of 21 patients. No secondary rise was found as had been the experience with hypertonic solutions of sodium chloride and dextrose. The effect of urea in reducing intracranial pressure is present despite bilateral nephrectomy in the monkey(4). The specific role of the choroid plexus in formation of cerebrospinal fluid is not understood. To gain insight into the role of this organ, the activity of 7 representative enzymes has been measured in the choroid plexus of the cat(5). This tissue was approximately one-third to one-half as active as liver and kidney and more active than most areas of the brain in specific reactions. Our findings do not allow us to conclude that this structure is responsible for production of CSF, but its anatomical and enzymatic properties strongly suggest that it may play a role in elaboration of this fluid. Since urea has a profound effect upon intracranial pressure, it became of interest to study the effect of this drug upon selected enzymatic activities of the choroid plexus.

*Materials and methods.* All studies were done on the cat. The animals were anesthetized with intravenous nembutal, 30 mg/kg, and the animal placed in the prone position. The cisterna magna was entered with a fine 25 gauge needle attached to polyethylene catheter connected to a pipette clamped in horizontal position and graduated in hundredths of a milliliter. Observations of the CSF flow were made every 10 minutes. After urea had been given intravenously, observations were continued for 3 hours. At end of experiment, the animal was sacrificed, and a sample of blood, a kidney, and entire brain were removed. The choroid plexus along with

TABLE I. Biochemical Activity of Cat Tissues.

| Tissue | Choroid plexus | Kidney | Blood | |
|---|---|---|---|---|
| Alkaline phosphatase | 1767 ± 96 (26)† | 3720 ± 804 (7) | | μmoles p-nitrophenol phosphate, split/hr/g |
| *Idem*, after I.V. urea (1 g/kg body wt) | 1311 ± 295 (8) | 3763 ± 1001 (8) | | *Idem* |
| Succinic dehydrogenase | 5760 ± 380 (13) | 18540 ± 1600 (8) | | μl/hr/g |
| *Idem*, after I.V. urea | 6863 ± 909 (3) | 17567 ± 1689 (4) | | *Idem* |
| Carbonic anhydrase | 179* ± 12 (56) | 100.1 ± 12.3 (12) | 450 ± 9.0 (74) | Ashby units/g tissue |
| *Idem*, after I.V. urea | 175* ± 15.8 (4) | 141.8 ± 29.7 (4) | 308 ± 74 (4) | *Idem* |

* Corrected for blood.

† No. of animals in parentheses.

sample of kidney were then dissected and immediately studied for its metabolic activity. Three enzymes were selected: alkaline phosphatase, found in high concentrations in walls of blood vessels, and succinic dehydrogenase and carbonic anhydrase, found previously to be in high concentrations in the ependymal layer. Analysis of tissues for activities of alkaline phosphatase, succinic dehydrogenase, and carbonic anhydrase was carried out as described previously(5).

*Results.* Table I illustrates no major differences in activity of these enzymes of the choroid plexus and kidney under the influence of intravenous urea, despite the fact that this drug promotes reduction of CSF flow and presumably pressure in the cat since there was no hemo-dilution as measured by hemoglobin analysis. The marked reduction of blood carbonic anhydrase values after urea treatment cannot be accounted for. Reduction in CSF flow is comparatively shortlived (Fig. 1), and our experiments showed the original flow rate was resumed generally within 40 minutes although some cases might be inhibited in flow rate for as long as 80 minutes. Once the original flow rate has been reestablished, intravenous urea may again have the same effect on flow rate.

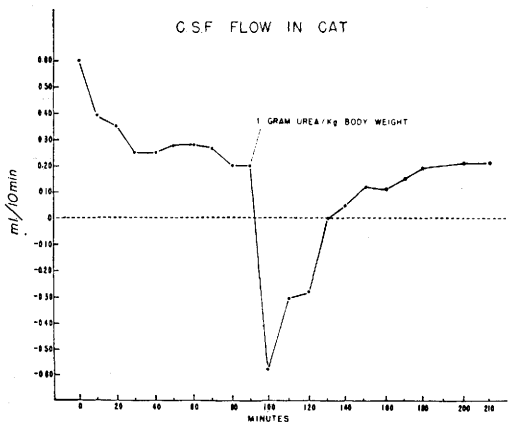


FIG. 1.

*Discussion.* Urea has been known to be a very effective diuretic but its effects in reducing intracranial pressure are not due to this phenomenon since bilateral nephrectomy in the experimental animal does not interfere with this effect in reducing pressure(4). Urea does not penetrate the blood-brain barrier as readily as it does other tissue(4) and it is conceivable that its effect may be due entirely to a difference of osmolarity between blood and cerebrospinal fluid. However, this study illustrates that enzyme activity of the choroid plexus is unaltered following intravenous urea therapy. From previous studies, however, we have been unable to conclude that CSF flow is dependent on enzymatic activity of the choroid plexus. It is quite possible that flow of CSF during urea therapy is unaltered and only absorption is changed.

*Summary.* Intravenous urea has been administered in cats. The flow rate of CSF is radically reduced but the activity of 3 enzymes of the choroid plexus (alkaline phosphatase, succinic dehydrogenase, and carbonic anhydrase) is unaltered.

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## Blood Thromboplastin: Its Preparation and Properties.* (25100)

THEODORE H. SPAET AND JOSE CINTRON
*Division of Laboratories, Montefiore Hospital, N. Y. City*

The concept of blood thromboplastin has been accepted by most workers in the clotting field since publication by Biggs and associates (1) of their classical studies on initial stages of blood coagulation. Bergsagel and Hougie (2) have shown that an activity corresponding to that of whole blood thromboplastin can be sedimented on platelets incubated in serum and recalcified plasma reagent, and they designated this activity "product 2." Similar activity has been sedimented on crude cephalin by Streuli(3); the present study deals with properties of such a thromboplastic preparation.

*Materials and methods.* Thromboplastin generation reagents were prepared as previously described(4), except that dilutions of all reagents were made with isotonic saline brought to pH 7.3 with imidazol buffer. Plasma reagent of human origin was diluted to 20% and bovine to 10%. The basic steps in preparation of blood thromboplastin were adapted from the method of Bergsagel and Hougie(2) as follows: one volume each of plasma reagent, serum reagent, and 0.025 M $CaCl_2$ were mixed and allowed to incubate at 37°C for 15 minutes. The clot which invariably formed was carefully removed, and an additional volume of cephalin reagent was added. Another 5 minutes incubation at 37°C gave a thromboplastin reagent which clotted recalcified human substrate plasma in 9-11 seconds. This was immediately centrifuged for 30 minutes and about 20,000 rpm at 4°C in an International PR-2 Refrigerated Centrifuge equipped with multi-speed attachment. The supernatant was discarded, and the sediment was resuspended in buffered isotonic saline containing 0.025 M $CaCl_2$. The mixture was again centrifuged as before, and the procedure repeated to give 2 washings. The last supernatant had no demonstrable thromboplastic activity. The final sediment was suspended in a volume of buffered calcium-saline equal to ¼ the volume of original generating mixture. By subsequent dilution this reagent was adjusted so that it clotted substrate human plasma in about 12 seconds. It was found that "cephalin" preparations from human brain or platelets(5) gave identical results. Plasma deficient in factor V was prepared by aging oxalated human plasma at 37°C for 3 days; plasma deficient in Stuart factor was kindly supplied by Dr. J. B. Graham from University of North Carolina.

*Results.* Blood thromboplastin obtained in the sediment was highly active, and had clotting properties described by others in whole thromboplastin generation mixtures(6). The reagent clotted normal, factor V-deficient, and Stuart factor-deficient plasmas equally well thereby demonstrating properties different from those of tissue thromboplastin (Table I). Blood thromboplastin preparation failed to clot 0.5% concentrations of Cohn's fraction I (Cutter) or substrate plasma with 0.1 cc of 0.38% sodium citrate substituted for calcium reagent, indicating absence of demonstrable thrombin activity.

TABLE I. Clotting Times in Seconds of Substrate Plasmas.

| | Thromboplastin | |
|---|---|---|
| | Tissue | Blood |
| Normal | 12 | 12 |
| Factor V—deficient | 37 | 13 |
| Stuart factor—deficient | 59 | 13 |

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