losses. (3) Application of low temperature (– 79°C) in the course of substitution seems to be justified.

Résumé. Les auteurs ont constaté que la température a une influence sur la diminution du contenu en azote et en phosphore et sur celle de la masse déséchée après l'usage de méthanol au cours de la congelation-dissolution (freeze-substitution) des tissus. Ces diminutions sont moins marquées à la température de – 79°C. Après l'usage d'acétone, les diminutions atteignent leur minimum.

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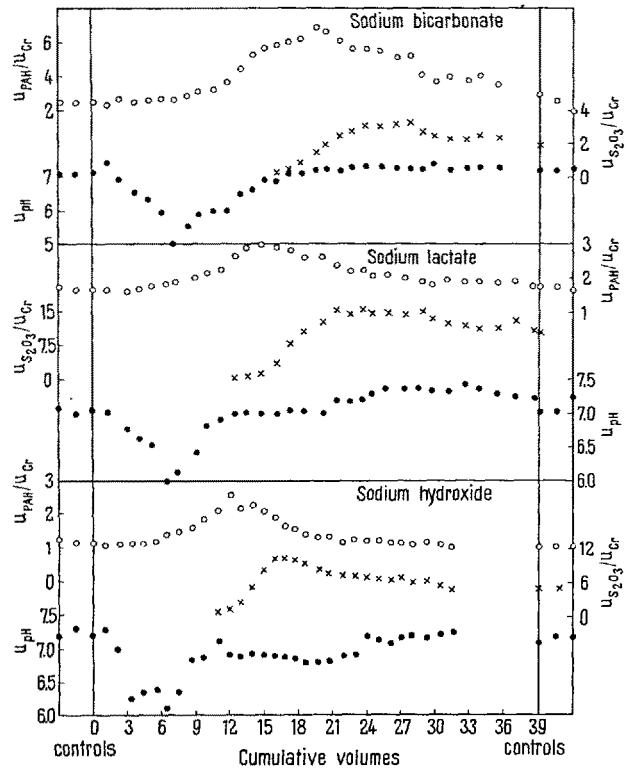
Stop-Flow Studies of Alkaline Urines

When an alkaline urine is produced, it has been found that the pCO_2 of the final urine may exceed that of the arterial blood by a considerable margin[1,2]. To explain this phenomenon, PITTS and LOTSPEICH[1] suggested that during formation of an alkaline urine, the distal tubule continued to secrete hydrogen ions in exchange for sodium ions, that such secretion led to the conversion of HCO_3^- to H_2CO_3, and that the delayed conversion of H_2CO_3 to CO_2 and water leads to a high urinary pCO_2. The authors are aware of no experiments designed to test this hypothesis directly. One of its predictable consequences is that acidification occurs in a distal segment despite the production of a final alkaline urine. This paper reports that such acidification can be demonstrated by means of the stop-flow technique[3].

Male 15 kg dogs were anesthetized with nembutal. The ureters were exposed, and catheters were inserted to the pelvis, and the free ends of the catheters brought out through flank incisions in preparation for carrying out stop-flow experiments to localize sites of pH change. In some dogs, either isosmotic $NaHCO_3$ or Na lactate solution containing PAH was infused into one femoral vein at a rate fast enough (3–6 ml/min) to cause production of a frankly alkaline urine. The remaining animals to be considered in this study were infused with NaOH by way of a catheter inserted into the jugular vein, and forced deeply enough so that its opening was at the expected level of the superior vena cava. After the urine had become adequately alkaline, infusion of 20% mannitol without any added salt was begun while continuing infusion of the alkalinizing solution. Sodium chloride was omitted since it was found that the urine pH would drop to the acid range if the mannitol solution contained salt. When urine flows became greater than 6 ml/min, a 6 min stop-flow experiment was run. The urine samples were collected manually and immediately covered with mineral oil to minimize pH changes. Because of the ease of analysis, S_2O_3 was used as a glomerular marker and injected 30 sec before the end of uretral clamping. PAH was used as a proximal marker.

The Figure shows typical results of experiments carried out by each of the methods utilized for inducing urinary alkalinization. In all experiments it was found that the pH of the urine coming from distal sites became acidified, even though the free flow urines before diureses never had a pH lower than 7.6 and ranged up to 8.5. The Table summarizes pertinent data on all experiments. No correlation was indicated between the distal pH drop, and free flow urinary pH or nature of the alkalinizing salt. No clear cut evidence for the locus of alkalinization was obtained.

It is assumed that the establishment of concentration gradients under stop-flow conditions are reflections of processes which occur in the elaboration of urine under free flow conditions. If such is indeed the case, then it may



Graphical representation of single experiments where alkalinization was produced by infusion of the salt indicated. Proximal regions of the nephron population are indicated by the peaked portion of the PAH curve. New glomerular filtrate is indicated by the rise in urinary S_2O_3 concentration. In all sections of the Figure the open circles show the PAH ratios, the crosses the S_2O_3 ratios, and the closed circles the urinary pH.

Magnitude of distal pH change in dogs loaded with an urinary alkalinizing salt

Salt	No. of experiments	Urinary pH[a] no diuresis	Urinary pH diuresis	Change distal pH
$NaHCO_3$	3	7.6–8.5	7.1–7.5	0.9–2.3
Na lactate	4	7.7–8.1	7.2–7.6	0.9–1.2
NaOH	4	7.9–8.2	7.0–7.4	0.8–1.3

[a] Numbers show ranges of the data.

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[2] C. RYBERG, Acta physiol. scand. *15*, 123 (1948).
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be concluded that a distal site continues to secrete acid during metabolic induction of urinary alkalinization. This observation is consistent with the hypothesis that at least a fraction of distal sodium reabsorbtion takes place by way of an exchange for hydrogen ions and therefore with the hypothesis advanced by PITTS and LOTSPEICH[1].

Although, no clear cut site of alkalinization could be detected in these studies, it is suggested that these data are consistent with proximal alkalinization. Such a process could be obliterated by the distal acidifying action as the proximal urine passed this site after release of the ureteral clamp[4]. Since the tubule is relatively permeable to CO_2, it is recognized that alkalinization could occur by concentrating the urine and increasing the ratio of HCO_3^- to H_2CO_3. Examination of the stop-flow curves show that the process of acidification takes place rather far distally, and it is considered unlikely that alkalinization secondary to concentration more distal than the locus of acidification is the main mechanism involved in producing high urinary pH. Such a process could occur, however, at the proximal level. A process of proximal alkalinization would be a corollary to that of proximal acidification demonstrated by GOTTSCHALK et al. under certain conditions[5].

The fact that it was not found possible to maintain a urinary pH above 7 when mannitol containing NaCl was infused is in agreement with the results reported by VAN SLYKE and EVANS[6]. They found that loading of normal dogs with NaCl and glucose induced a fall in pH of the urine. The acidification was attributed to be a consequence of reduction of plasma HCO_3^- concentration secondary to dilution. Examination of their data, however, shows that a considerable drop in pH was found before the drop in plasma HCO_3^- became significant. Furthermore, it would be difficult to explain why mannitol containing NaCl should cause more dilution than mannitol alone in these experiments. It may be that increasing the sodium load is responsible for increasing tubular exchange of H^+ for Na^+[7].

Zusammenfassung. Bei Hunden, die wegen Infusion von $NaHCO_3$, Na-Laktat oder NaOH alkalischen Harn produzierten, wurden «stop-flow»-Versuche durchgeführt. Es konnte gezeigt werden, dass unter diesen Umständen distal eine Ansäuerung des Tubulusharns stattfindet, so dass die Alkalisierung in den proximalen Abschnitten stattfinden muss.

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Mechanism of Action of Cocaine and Amphetamine in the Brain

The central stimulant action of cocaine is to a great extent similar to that of the amphetamines. Cocaine and amphetamines cause similar changes in the EEG pattern, while evoking alertness and suppressing appetite[1] to a similar degree.

The amphetamines are structural analogues of catecholamines (arterenol and dopamine); there exists a rigid structure-activity relationship[2]. This supports the suggestion that amphetamines may mimic the action of catecholamines on specific receptors in the brain[3] and that they may therefore have a direct arterenergic mechanism of action.

The central action of cocaine seems to be related to its sympathetic action since its stereo-isomer pseudococaine, although having identical local anaesthetic properties with cocaine, is devoid of peripheral sympathetic activity and of a central stimulant action[4]. Furthermore, the central stimulant action of cocaine and that of amphetamine is selectively antagonized by sympatholytic drugs such as dibenzyline and piperoxane. There is no structural relationship between cocaine and catecholamines and amphetamines so that a direct catechol-like action seems improbable. Alternatively an indirect arterenergic action might underlie the action of cocaine in the brain, that is, an action by release of catecholamines. Experiments will be conducted to test this hypothesis.

Reserpine causes a release of catecholamines (CA) and finally a depletion of long duration[5]. This would imply that indirect arterenergic drugs that act by releasing CA, should no longer be active after depletion of CA by reserpine, whereas those directly occupying the specific receptors ought still to exert their normal effect.

Spontaneous motor activity of groups of two mice was measured with the cumulative recording procedure[6]. About 1 or 2 h after the mice had been placed in the cage and their exploratory behaviour had subsided, they were injected either with *d*-amphetamine (5.62 and 10 μMol/kg in succession) or *l*-cocaine (31.6 and 56.2 μMol/kg) (see Figure 1a and 2a). Cocaine induces a similar increase in motor activity as amphetamine, which is, however, of short duration. Amphetamine in an equally active dose has an action 2–4 times longer. The animals used in this type of experiments were injected with 0.5 mg/kg reserpine twice a day over 3 consecutive days starting 5 days after the previous test doses of amphetamine or cocaine. On the fourth day the same doses of cocaine and amphetamine were administered as given previously and again motor activity was recorded (see Figure 1b and 2b). Similar results were obtained when both amphetamine and cocaine were injected in the same group of two mice. For average results see Table.

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