treten lassen, gingen wir zu vergleichenden Versuchen über, die wir in Carrelschalen mit zusätzlicher Begasungseinrichtung vornahmen[14]. Als malignes Testmaterial verwendeten wir Jensen-Sarkom der Ratte, das 11 Tage nach der Beimpfung exstirpiert und in Form von dünnen Gewebestückchen (etwa 1 mm²) zunächst 5 Tage lang in üblicher Weise auf festem Plasmaboden angezüchtet wurde. Vom 6. bis 9. Tag wurde dann mit Reinststickstoff, dem etwa 5% O_2-freies CO_2 beigemischt waren, kontinuierlich begast. Im Verlauf der anaeroben Versuchsperiode erfolgte die Fütterung (ebenfalls streng anaerob) in Versuchsreihe I weiterhin mit normalem Nährsubstrat (2 Teile Tyrodelösung, 2 Teile Pferdeserum, 1 Teil Hühnerembryonalextrakt), in Versuchsreihe II dagegen mit Krebskochsaft-Nährlösung, die sich von der normalen lediglich dadurch unterscheidet, dass die Tyrodesalze hierbei nicht in Wasser, sondern in der gleichen Menge Krebskochsaft gelöst werden.

Am 9. Versuchstag, unmittelbar nach Abschluss der Begasung, wurden die Kulturen beider Versuchsreihen aus den Carrelschalen herausgenommen. Die erste Hälfte der Explantate prüften wir auf Lebensfähigkeit mit Trypanblau (Verdünnung 1:5000), die andere Hälfte präparierten wir zwecks Untersuchung auf Mitosen (Fixierung mit Kaliumbichromat-Formalinlösung; 3wöchige Einbettung in Celloidin-Paraffin). Alsdann wurden die angefertigten Gewebeschnitte zur Mitosen-Bestimmung einmal mit Hämatoxylin-Eosin[15] angefärbt und zweitens der Feulgen-Reaktion unterzogen.

Auf Grund der Trypanblau-Färbung und des Mitosen-Nachweises gelangten wir zu folgendem Untersuchungsergebnis:

Versuchsreihe I: intensive Blaufärbung der peripheren Zellen, keine Mitosen;

Versuchsreihe II: keine Färbung der peripheren Zellen, Mitosen in mehreren Bezirken.

Der Ausfall obiger Vergleichsversuche gibt eindeutig zu erkennen, dass – unter strikter Fernhaltung von Sauerstoff – die mit Krebskochsaft behandelten Krebszellen resistenter sind als die unbehandelten. Unter dem Einfluss der in den Krebsdekokten von uns nachgewiesenen energiefreisetzenden Cofaktoren bleibt offensichtlich die Lebensfähigkeit der Krebszellen und ihr Vermögen zur Mitosenbildung länger erhalten als ohne Eingreifen dieser Akzessorien. Das vorliegende positive Ergebnis veranlasste uns, zu Untersuchungen an Einzell-Kulturen überzugehen und den Transplantationstest einzubeziehen.

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Summary

Cancer cells treated in anaerobic tissue-culture with tumour-decocts keep their vitality and their ability of building mitosis longer than untreated ones. From this it follows that electrontransferring cofactors found in tumour-decocts excite an anoxygenic energy-effect also on cancer tissues.

oxygenen Reaktivierung im Anabiosestadium besteht darin, dass sie nur bei Gärungszellen, nicht aber bei Atmungszellen (Q_{O_2}: $Q_{O_2} \approx 1$) eintritt.

[14] F. Windisch und W. Heumann sowie H. Kriegel und A. Graffi (Gemeinschaftsarbeit), Z. Naturforsch. *8b*, 673 (1953).

[15] Hämatoxylinlösung nach Delafield und Romeis, *Mikroskopische Technik* (Leibniz-Verlag, München 1948), und zwar § 669.

Effects of Acute Hypochloremia on Glomerular Filtration Rate and Electrolyte Excretion in the Dog

Acute hypochloremia was induced in dogs by the use of an artificial kidney[1]. During a priming stage, plasma chloride concentration was acutely decreased by dialyzing the blood against an isotonic chloride-free rinsing fluid where NaCl was replaced by $NaHCO_3$ or $NaNO_3$ (Table I). Subsequently, a steady hypochloremic state was maintained by a second bath containing electrolytes in concentrations similar to those of the dog plasma at the end of the priming stage. With this method it was possible to study the effects of hypochloremia without hyponatremia and with or without alkalosis on the glomerular filtration and electrolyte excretion rates (Fig. 1 and 2).

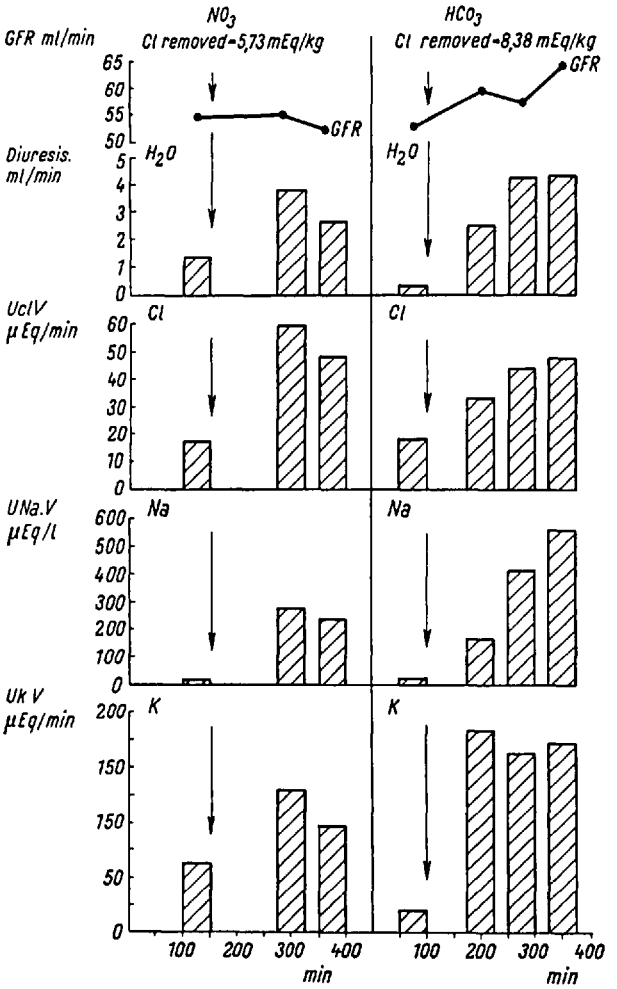

Fig. 1.—Glomerular filtration rate and electrolyte excretion changes observed in same experiments as in Figure 2.

3 experiments were performed with $NaNO_3$ and 6 with $NaHCO_3$. The following results were obtained during 12 standard 10 min-clearance periods for each experiment.

(1) *Glomerular Filtration Rate*[2]. Hypochloremia, with

[1] We are much indebted to Dr. R. P. Herwick, from Baxter Laboratories, Morton Grove, Ill. (U.S.A.), for supplying the Kolff's coil kidney units used in these experiments.

[2] The experiments involving a drop of blood pressure, as is often observed during hemodialysis in dogs, have been omitted from our results.

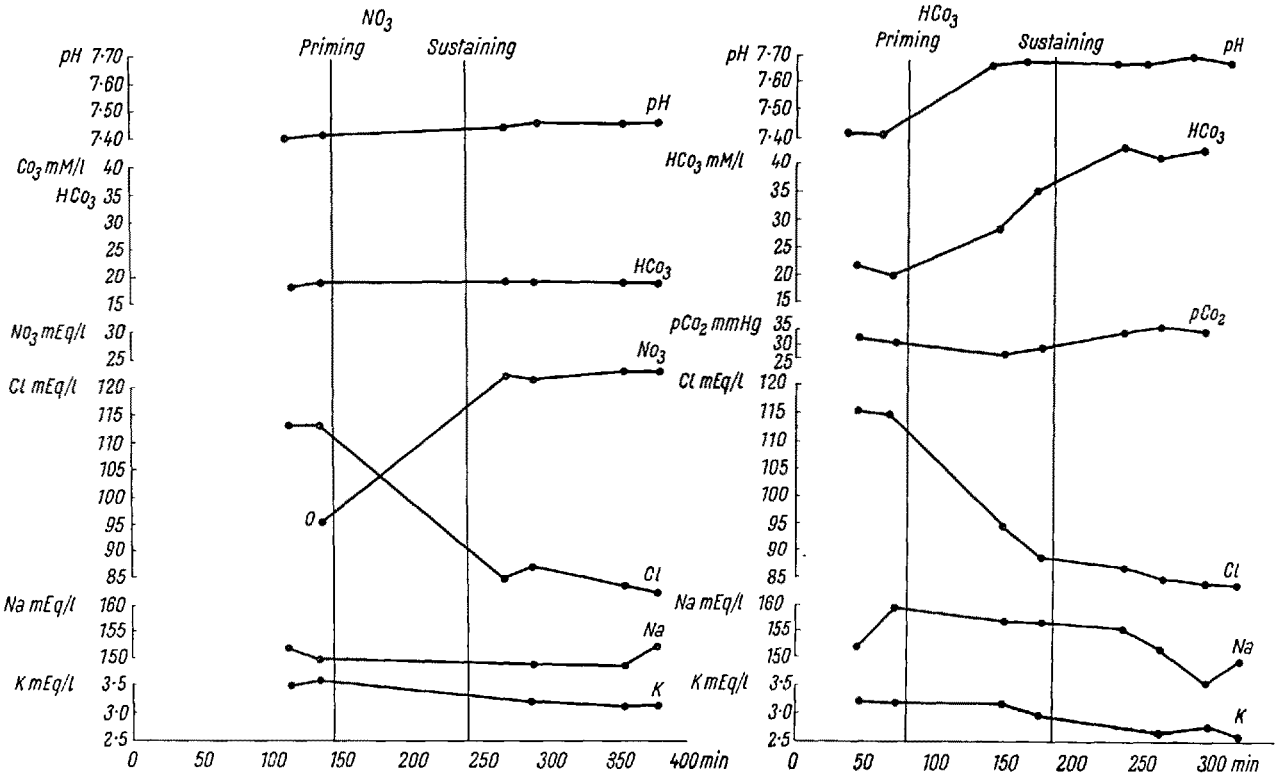

Fig. 2.—Blood changes during NO_3 (left) and HCO_3 (right) induced hypochloremia. The vertical lines indicate the beginning of each type of bathes used.

or without alkalosis, does not lower the glomerular filtration rate (Table II).

(2) *Sodium Excretion.* Although there was no significant change in the sodium glomerular filtered load during these experiments, the excretion of sodium increased markedly in both types of hypochloremia.

The ratio of excreted Na/filtered Na rose from a mean control value of 0·2 up to 4·5% when the chloremia was decreased from 117 to 84 mEq/l.

Table I
Composition of Bathing Fluids

Bathing Fluids	Milliequivalents per l						
	Cl	NO_3	HCO_3	Na	K	Ca	Mg
Normal	120	—	30	142	4	2·5	1·5
NO_3 Priming	8	115	30	145	4	2·5	1·5
Sustaining	80	143	30	145	4	2·5	1·5
HCO_3 Priming .	8	—	145	145	4	2·5	1·5
Sustaining	80	—	73	145	4	2·5	1·5

(3) *Potassium Excretion.* Potassium excretion was also raised during both types of experiments. In NO_3-induced hypochloremia, the ratio excreted K/filtered K rose from 18 up to 65% as the plasma chloride concentration fell from 118 to 84 mEq/l. Potassium excretion was much more increased in hypochloremic alkalosis where the excreted K/filtered K ratio increased proportionally to the blood pH (Fig. 3), as has been previously shown by FRANGLEN *et al.*[3].

[3] G. T. FRANGLEN, E. MCGARRY, and A. G. SPENCER, J. Physiol. *121*, 35 (1953).

(4) *Chloride Excretion.* Despite the marked reduction of the chloride glomerular filtered load, chloride excretion was usually increased in both types of hypochloremia. The ratio of excreted Cl/filtered Cl rose from 0·2 up to 1·4% in the nitrate experiments and from 0·7 to 1·0% in hypochloremic alkalosis.

(5) *Bicarbonate Excretion.* Although bicarbonate excretion was regularly increased in hypochloremic alkalosis, it never reached the values observed during 4 control experiments where a 6% $NaHCO_3$ solution was infused

Table II
Effect of Hypochloremia on Glomerular Filtration Rate

Experiment No.	Plasma chloride mEq/l		Glomerular Filtration Rate ml/min**	
	Control	Hypo-chloremia	Control	Hypo-chloremia
1*	118	89	54·6	55·5
2*	121	76	90·8	89·8
3*	115	87	71·2	68·8
4	116	92	76·2	75·5
5	116	92	53·1	60·4
6	118	76	64·1	68·0
7	118	80	43·4	59·0
8	115	85	69·9	79·0
9	116	79	81·3	95·0
Means	117	84	67·2	72·3

* NO_3-induced hypochloremia.
** Each value represents the mean of three successive creatinine clearance periods.

in dogs submitted to hemodialysis against a normally balanced bathing fluid. In these latter experiments, not involving hypochloremia, the usual threshold value for bicarbonate was obtained: from 26 to 28 *mM* of bicar-

bonate reabsorbed per liter of glomerular filtrate[4]. In contrast to this, our dogs in hypochloremic alkalosis showed bicarbonate threshold values rising parallel to the

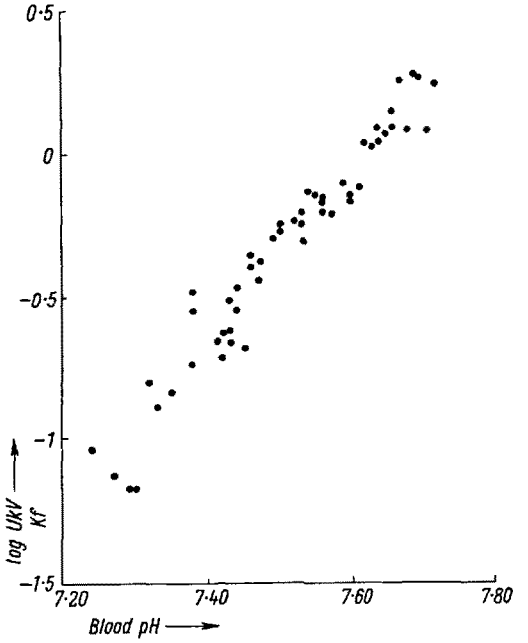

Fig. 3.—The ratio of excreted K/filtered K in relation to blood pH in hypochloremic alkalosis.

plasma bicarbonate concentration (Fig. 4), such as has been observed by RELMAN *et al.*[5], BRAZEAU and GILMAN[6], and DORMAN and SULLIVAN[7] in respiratory acidosis and by ROBERTS *et al.*[8] in potassium depletion. A similar increase in bicarbonate reabsorption was observed when a 6% $NaHCO_3$ solution was infused during NO_3-induced hypochloremia.

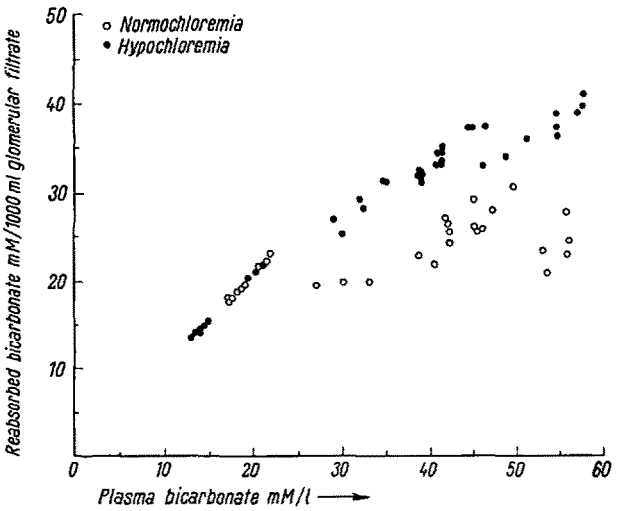

Fig. 4.—Bicarbonate reabsorption during hypochloremic alkalosis (black circles) and during 6% $NaHCO_3$ infusion (open circles). The values under 25 mEq/l represent data obtained during the control periods.

These findings demonstrate a definite competition between bicarbonate and chloride in tubular reabsorption. This phenonemon was first described by PITTS and LOTSPEICH[4] and more recently by HILTON *et al.*[9] who studied the effect of increased plasma chloride concentration on bicarbonate reabsorption.

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Résumé

La chlorémie du chien a été abaissée à l'aide d'un rein artificiel en utilisant des liquides de dialyse où une certaine quantité de chlore était remplacée par du bicarbonate ou du nitrate. Dans ces conditions, l'hypochlorémie s'accompagne:

1° d'une conservation de la filtration glomérulaire;

2° d'un accroissement de l'excrétion du sodium, du potassium et du chlore;

3° d'une élévation du seuil d'excrétion du bicarbonate.

[4] R. F. PITTS and W. D. LOTSPEICH, Amer. J. Physiol. *147*, 481 (1946).

[5] A. S. RELMAN, B. ETSTEN, and W. B. SCHWARTZ, J. clin. Invest. *32*, 972 (1953).

[6] P. BRAZEAU and A. GILMAN, Fed. Proc. *12*, 19 (1953).

[7] P. J. DORMAN and W. J. SULLIVAN, Fed. Proc. *12*, 34 (1953).

[8] K. E. ROBERTS, H. T. RANDALL, H. L. SANDERS, and M. HOOD, J. clin. Invest. *34*, 666 (1955).

[9] J. G. HILTON, N. E. CAPECI, G. T. KISS, O. R. KRUESI, V. V. GLAVIANO, and R. WÉGRIA, J. clin. Invest. *35*, 481 (1956).

On the Participation of the Zona Glomerulosa on the Adrenal Response to Stress

The adrenal glomerulosa is considered to be the site of mineralocorticoid production, as was recently proved by HARTROFT and EISENSTEIN[1]. However, the physiological mechanism by which its function is regulated still remains obscure. The conception of the high independence of glomerulosa on pituitary control[2] disagrees with the observation of glomerulosa reaction taking place only in the presence of hypophysis[3] or the glomerulosa reaction on ACTH in hypophysectomized animals[4]. The experimental results, here presented, contribute to the closer elucidation of this problem.

White Wistar rats, males about 5 months old and 200 to 250 g in weight, were the object. The rats were stressed by a single intramuscular injection of 4% formaldehyde in the quantity of 0·3 ml/100 g, and were killed at intervals of 1 h and 3 h after the injection. The adrenals were fixed by 4% formaldehyde with 1% $CaCl_2$. The lipids of

[1] P. M. HARTROFT and A. B. EISENSTEIN, Endocrinology *60*, 641 (1957).

[2] H. W. DEANE and R. O. GREEP, Amer. J. Anat. *79*, 117 (1946). – H. W. DEANE, J. H. SHAW, and R. O. GREEP, Endocrinology *43*, 133 (1948).– E. KNOBIL, A. MORSE, and R. O. GREEP, Anat. Rec. *121*, 324 (1955).

[3] K. KOVÁCS, É. HORVÁTH, B. M. KOVÁCS, G. S. KOVÁCS, and G. PETRI, Arch. int. Pharmacodyn. *108*, 170 (1956). – K. KOVÁCS, B. M. KOVÁCS, G. S. KOVÁCS, and G. PETRI, Naturwissenschaften *44*, 241 (1957). – A. NAGY, A. OLAH, and S. KARADY, Nature *180*, 1481 (1957).

[4] B. C. WEXLER, A. P. RINFRET, A. C. GRIFFIN, and H. L. RICHARDSON, Endocrinology *56*, 120 (1955). – J. D. LEVER, Endocrinology *58*, 163 (1956).