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

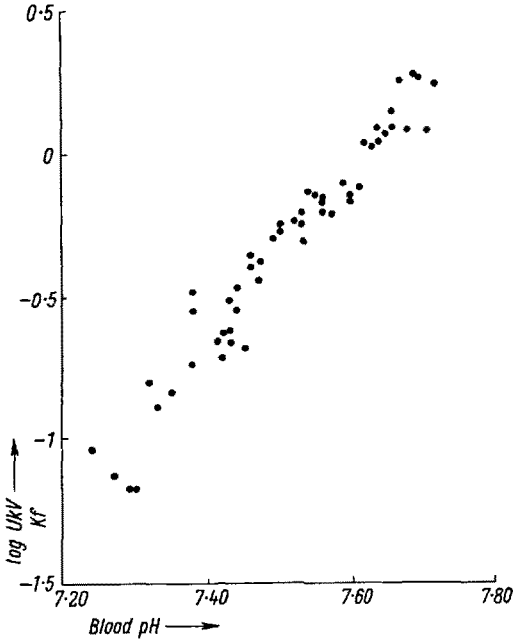


Fig. 3.—The ratio of excreted K/filtered K in relation to blood pH in hypochloremic alkalosis.

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.

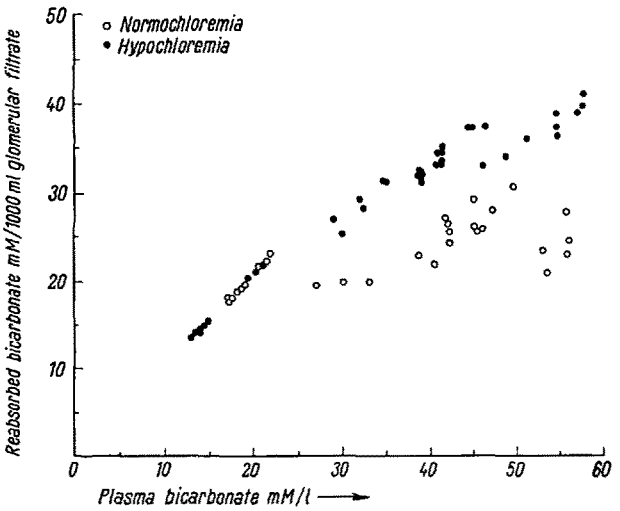


Fig. 4.—Bicarbonate reabsorption during hypochloremic alkalosis (black circles) and during 6% $NaHCO_3$ infusion (open circles). The values under 25 mEq/l represent data obtained during the control periods.

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.

CH. TOUSSAINT, M. TELERMAN, and P. VEREERSTRAETEN

Laboratoire de Médecine expérimentale, Université Libre de Bruxelles et Fondation Médicale Reine Elisabeth, Bruxelles (Belgium), June 30, 1958.

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.

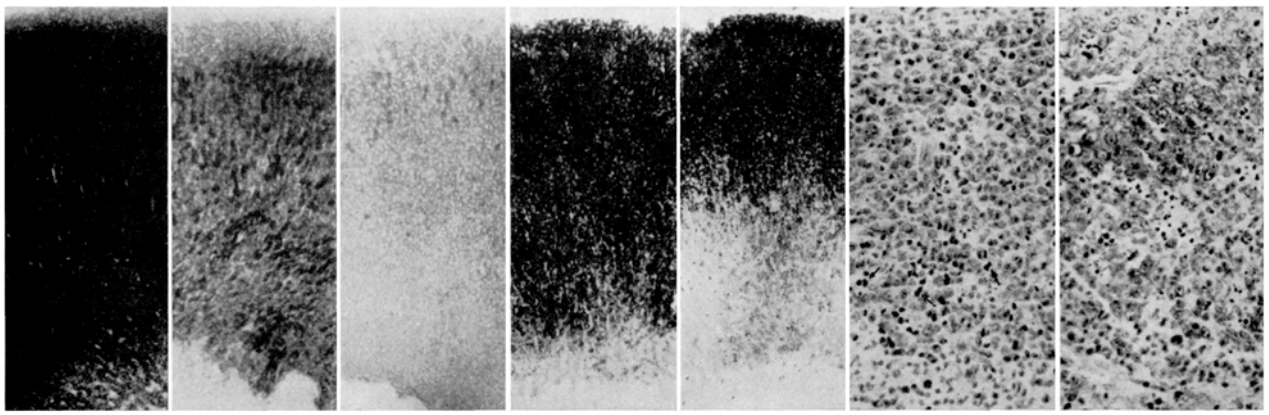
White Wistar rats, males about 5 months old and 200 to 250 g in weight, were the object. The rats were stressed by a single intramuscular injection of 4% formaldehyde in the quantity of 0·3 ml/100 g, and were killed at intervals of 1 h and 3 h after the injection. The adrenals were fixed by 4% formaldehyde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



1 2 3 4 5 6 7

Fig. 1–3.—Series A. The adrenal cortex of the non-stressed rat (Fig. 1) and 1 h (Fig. 2) and 3 h (Fig. 3) after the formaldehyde injection. The progressive lipid depletion from the fascicular as well as glomerular layer can be observed. Sudan black B. × 80.

Fig. 4–5.—Series B. The adrenal cortex of the non-stressed rat (Fig.4) and 3 h after the formaldehyde injection (Fig. 5). Zona fasciculata of non-stressed rat of this series contained less lipids than those of series A. In the third hour after the formaldehyde injection, the lipids disappeared from deeper layers of the fasciculata; zona glomerulosa remains non-influenced. Sudan black B. × 80.

Fig. 6–7.—The centre of spleen lymphatic nodule of the rat of series A (Fig. 6) and series B (Fig. 7) in the third hour after the beginning of stress. The swelling of lymphoid tissue and a marked lymphocyte disintegration took place only in the nodules of series B (Fig. 7). The nodules of series A were moderately penetrated by erythrocytes (↑). Azur II-Eosin. × 295.

the frozen sections (15 μ) were stained with Sudan black B and with Oil red O. Moreover, the number of leucocytes, their differential count, and the absolute number of eosinophils were determined. The spleen and the thymus were made up by histological technique. Paraffin sections (5 μ) of these organs were stained with Azur II-Eosin[5].

Series A, used on July 11th, containing 15 rats, was divided into trios injected by formaldehyde successively between 9 a.m. and 5 p.m. One rat of each trio was the non-injected control. In series B, treated on October 8th, the formaldehyde injection in 10 rats was carried out at 9 a.m. Twelve control rats were killed, partly at the beginning of this experiment, partly together with stressed rats.

Series A: As early as 1 h after formaldehyde injection, the lipids of the fasciculata as well as of the glomerulosa became markedly reduced (Fig. 2). The depletion of lipids affected the cell districts of fasciculata between which there could be found single cells or groups of cells containing the full amount of lipid droplets in their cytoplasm. In the glomerulosa, the lipids gradually disappeared, especially from the cytoplasm of subcapsular cells. 3 h after formaldehyde injection, the lipid discharge in the adrenal cortex was still more advanced (Fig. 3). Cells of the fasciculata preserving the original range of lipid droplets were scarce. In the glomerulosa the Sudan-positive material of subcapsularly localized cells completely disappeared; however, in deeper layers of this zone, some was preserved in small amounts here and there.

Series B: The adrenals of non-stressed rats of this series differed from those of series A by less lipid content in the fasciculata (Fig. 4). The lipid depletion involving exclusively the fascicular layer was discernible as late as 3 h after stress stimulus (Fig. 5). It is noteworthy that the glomerular layer remains unaffected at the same time. Furthermore, in distinction to the entirely depleted adrenal cortex in the series A, the disappearing of lipids was carried out only in the deeper layers of fasciculata. Therefore the zona fasciculata of the stressed rats of series B became relatively more abundant in lipids than those of series A at corresponding intervals.

Regarding the structure of lipid inclusions, it is worthy of note that in adrenals of non-stressed, as well as of stressed rats of series A, the lipids were deposited in cells both of glomerulosa and fasciculata in the form of very fine droplets; on the other hand, in series B in the form of coarse vacuoles.

Both series of rats differed in their initial level of leucocytes which amounted to 12,900 ± 4,205 in series A and to 7,750 ± 1,600 in series B. The difference was statistically significant, $p < 0{\cdot}01$. Similarly the difference in the initial lymphocyte level amounting to 10,404 ± 3,252 in series A, whereas only to 5,717 ± 1,845 in series B, was statistically significant, $p < 0{\cdot}002$. The differences in values of neutrophils and eosinophils were not significant. Up to the third hour after formaldehyde injection, the lowering of lymphocytes and eosinophils, and the disintegration of lymphocytes in the thymus cortex was noticed in both series without any apparent difference. Different results were gained when qualifying the stress changes in the light centres of the lymphatic nodules of the spleens. In series A, the disintegration of lymphocytes was nearly absent (Fig. 6). On the other hand, in series B, swelling of the nodular lymphoid tissue, lacking in series A, and marked disintegration of lymphocytes, was found (Fig. 7). In the centres of spleen nodules of non-stressed rats in series A, the reticular cells predominated over large and medium-sized lymphocytes; on the other hand, the centres in series B contained a well-formed zone of large and medium-sized lymphocytes and a zone of reticular cells.

Discussion. Stress response of the adrenal cortex differed in both series of rats. However, even the starting state of the rats of both series was different as judged by the blood lymphocytes level, by the activity of centres of spleen lymphatic nodules and by the lipid content of the zona fasciculata. Regarding the low lipid content of fasciculata, we may suppose a high initial peripheral glucocorti-

[5] M. BLOCK, V. SMALLER, and J. BROWN, J. Lab. clin. Med. *42*, 145 (1953).

coid level in rats of series B. Therefore the fasciculata of their adrenals might respond to stress in a less intensive way than those of series A, where, on the contrary, a lower initial glucocorticoid level could be supposed. The lipid depletion of glomerulosa took place only in the case of intensive stress reaction of fasciculata. Deducing from SELYE's[6] conception of reciprocal function of gluco- and mineralocorticoids, we may suppose the reaction of glomerulosa in series A to compensate the overshooting stress reaction of the fasciculata. The stimulus to the mineralocorticoid secretion probably came from the tissue changes, especially from the changes in their physical chemical state, caused by glucocorticoids. Our view is supported by an observation of a marked swelling of spleen lymphoid tissue after the hydrocortisone injection in adrenalectomized rats[7], which was far more developed than the lymphoid tissue swelling in series B. This would be expected because the supposed abolishing action of mineralocorticoids did not entirely take place in adrenalectomized ones.

The factors determining the neurohumoral state of both series of rats could not be defined more precisely. We consider the influence of the diurnal cycle itself to be almost improbable. Beside it, both the season and the temperature of the surroundings must exercise some influence.

M. HILL, M. POSPÍŠIL, and K. DVOŘÁK

Department of Histology and Embryology, Faculty of Medicine, University of Brno and Biophysical Institute of the Academy of Sciences in Brno (Czechoslovakia), June 3, 1958.

Zusammenfassung

Zwei Serien Ratten wurden durch Formalininjektion belastet. Die Zona glomerulosa der Nebennierenrinde reagierte durch Lipoidausscheidung nur in jener Serie, die durch hohe Lymphozytenzahl des Blutes, hohen Lipoidgehalt der Fasciculata, kleinere Aktivität der Lymphknötchen der Milz und auffallend intensive Stressreaktion der Fasciculata charakterisiert war. Bei Ratten, deren Glomerulosa ihre Lipoide ausgeschieden hatte, trat weder Schwellung noch Desintegration des lymphatischen Gewebes der Milz auf.

[6] H. SELYE and G. HEUSER, *Annual report on stress 1955–1956* (New York 1956).

[7] M. HILL, unpublished observation.

The Action of Cortisone on the Embryonic Cartilage and Muscle *in vitro*

Cortisone exerts an inhibitory action on the growth of rats[1] and produces marked abnormalities in the newly formed cartilage and bone[2]. These effects of the hormone could be derived from its known suppressing action on the development of all the elements of mammalian connective tissues[3]. Inhibition of growth by cortisone has also been shown in the chick embryo[4] and its anti-inflammatory effect on chorio-allantoic grafts[5]. The question has, therefore, been raised whether such effects could be attributed to a direct action of the hormone on bone growth or else are intermediate through some other endocrines or the general metabolism.

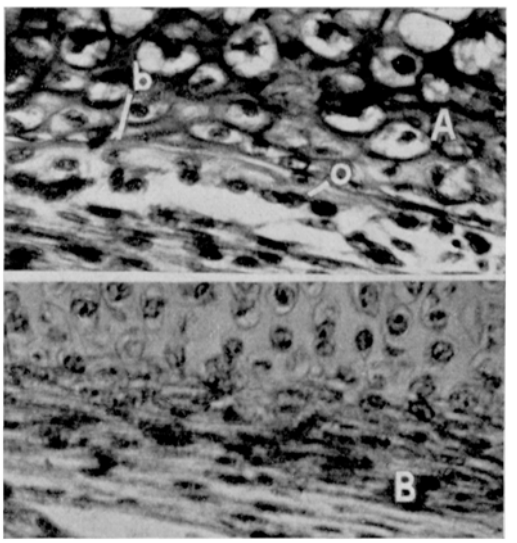


Fig. 1.—Longitudinal sections through femora of 6-day chick embryos, cultivated for 8 days: *A* – on horse serum-ascitic fluid medium and *B* – on a similar medium with cortisone added. H.-E. × 900, *o* = osteoblasts; *b* = bone material.

In the experiments reported here, the effect of cortisone on femora from 6-day chick embryos cultivated by the liquid medium-cellulose membrane procedure on a heterologous medium[6] were studied.

Cortisone* in the amount of 0·02 mg/ml was added to the liquid culture medium. Thus the action of the hormone was direct upon the cartilageneous rudiment of the femur, precluding interjacent factors. In total 32 cultures were grown, 16 on media containing cortisone throughout the whole period of experimentation and 16 without the hormone, as controls. Both femora of one embryo were cultivated in the different media, thus possible individual differences were excluded.

Results. The effects of cortisone were manifested in the retardation of growth of the whole rudiment, and in the bending of the explants which occurred more often in the treated cultures than in the controls. Histologically the untreated explants showed the typical developmental changes of the embryonic cartilage *in vitro* most exactingly described by Fell[7]. After the 8th day of cultivation the diaphyseal cartilage showed a marked hypertrophy (Fig. 1 A), characteristic of the stage preceding the penetration of the cartilage by the connective tissue of the periosteum *in vivo*. The cells were swollen into vesicles and the interstitial substance between them became thin and calcified. On the surface of the diaphysis a continuous layer of cells appeared suggesting a row of osteoblasts associated with the formation of osseous tissue (Fig. 1 A, *o*). A thin but distinct band of bone material was formed around the diaphysis (Fig. 1 A, *b*).

[1] B. B. WELLS and E. KENDALL, Proc. Staff Meet. Mayo Clin. *15*, 324 (1940).

[2] R. H. FOLLIS, jr., Proc. Soc. exp. Biol. Med. *76*, 722 (1951).

[3] CH. RAGAN, *Connective tissues* (First Conference, J. MACY, jr. 1950).

[4] D. A. KARNOFSKY, L. P. RIDGEWAY, and P. A. PATTERSON, Endocrinology *48*, 596 (1951). – G. L. SAMES and J. H. LEATHEM, Proc. Soc. exp. Biol. Med. *78*, 231 (1951). – H. SOBEL, Proc. Soc. exp. Biol. Med. *97*, 495 (1958).

[5] H. SOBEL, Bull. Res. Counc. Israel *4*, 249 (1954).

[6] H. SOBEL and H. LEURER, Exper. *14*, 213 (1958).

[7] H. B. FELL and R. G. CANTI, Proc. Roy. Soc. [B] *116*, 316 (1934).

* Cortisone-acetate in dry powder form was kindly supplied by CIBA AG., Basel.