Journal of Steroid Biochemistry, 1970, Vol. 1, pp. 111–120. Pergamon Press. Printed in Great Britain

FEEDBACK MECHANISM IN REGULATION OF ALDOSTERONE AND CORTISOL SECRETION

M. G. KOLPAKOV, S. G. KOLAEVA, P. M. KRASS, M. G. POLJAK, G. P. SOKOLOVA, G. S. CHUDNOVSKY, Yu. P. SHORIN and I. Sh. STERENTAL

Laboratory of Endocrinology of Natural Adaptations, Institute of Physiology, Siberian Branch of the Academy of Sciences of the U.S.S.R., Novossibirsk 90, U.S.S.R.

(*Received* 24 *February* 1969)

SUMMARY

The studies were carried out on dogs treated with high doses of cortisol (3 mg/kg/day for 10 days) or aldosterone (100 μg/kg/day for 5 days). Administration of exogenous cortisol decreases the cortisol production by an isolated adrenal gland sharply, and increases its aldosterone production. The plasma sodium and potassium concentration and renin-angiotensin system activity are unchanged. An administration of exogenous aldosterone suppresses aldosterone synthesis almost completely without affecting cortisol and corticosterone production. The sizes of nuclei and nucleoli in the glomerular zone cells remain unchanged. The juxtaglomerular granularity index (JGI) and renin content of the kidneys decrease sharply. Electrolyte concentration and exchangeable sodium are the same as in control. The unchanged Na_e at the time cumulative sodium balance was still strongly positive could result from the existence of a slowly exchangeable sodium pool the size of which would be selectively influenced by aldosterone. The effect of cortisol on the adrenals consists of two types of influence: the inhibitory one, by way of a feed-back mechanism, and a stimulating one, reflecting the general trophic effect of the hormone. A hypothesis suggests that the feedback in the effect of aldosterone is mediated through an increase of sodium concentration in the glomerular zone cells without affecting the RNA-dependent nucleolar apparatus which is possibly responsible for earlier stage of steroidogenesis.

INTRODUCTION

ACCORDING to present concepts, the regulation of secretion of corticosteroid hormones is ensured by an interaction of direct and feedback relations between the pituitary–adrenal and renal–adrenal systems.

The control of cortisol secretion is effected by a mechanism integrating the information which comes from stress influences and blood cortisol concentration. Any increase in blood cortisol concentration inhibits directly the "control mechanisms", i.e. the corticotropin-releasing factor–ACTH system, which leads to a decrease in cortisol secretion [1, 2].

No similar control mechanism reacting quickly to any change in blood aldosterone concentration has been found. Biglieri and Ganong[3] have shown that an intravenous infusion of aldosterone to anaesthetized acutely hypophysectomized dogs does not influence the aldosterone secretion rate whether the animal is sodium deficient or not. Supposing that anaesthesis and trauma could have thwarted the normal response of the control system, Blair-West *et al.*[4] carried out a study on non-anaesthetized moderately sodium-deficient sheep. The results of the experiments have corroborated the earlier observations[5] that a high peripheral blood aldosterone concentration has no direct inhibitory influence upon the aldosterone secretion rate. Having obtained negative results, the authors do not rule out the existence of a feedback relation in the control of the aldosterone

this effect is mediated through the parameter determining the renin–angiotensin secretion rate by the kidneys [6, 7], attention being paid to the fact that the aldosterone effect is only observed over prolonged intervals. An inhibition of renin secretion has been shown in primary aldosteronism [8, 9] but not after short-term infusions of aldosterone [9]. In long-term infusion of deoxycorticosterone (DOC) there was a decrease of renin content in kidneys [7] and of aldosterone secretion [10]. There are some reasons to suppose that the decrease in renin and aldosterone secretion could be a result of a long-term action of an elevated blood aldosterone concentration. The causal relationship between these processes and the feedback mechanism of aldosterone effect seems to be obscure.

In this study of dogs, the effects of large doses of cortisol and aldosterone upon the adrenal are compared.

MATERIAL AND METHODS

The experiments were carried out on mongrel male dogs, which during the experiment were kept in metabolic chambers.

In experiments with cortisol administration the preparation (hydrocortisone "Richter") was injected intramuscularly, (3 mg/kg a day) for 10 days. Dogs were fed food providing 60–70 mEq of Na and about 30 mEq of K a day. In experiments with aldosterone administration the drug (d,l-aldosterone "Serva") was injected subcutaneously in doses of 100 μg/kg/day for 5 days. These dogs were given a diet containing 70 mEq of sodium and 20 mEq of potassium a day. The control dogs were given a respective diet and injected with the respective solvents at the respective time.

The total of exchangeable sodium in the animals was determined on the basis of plasma specific activity 24 hours after the injection of ^{24}Na [11, 12].

The concentration of Na and K in food, urine and plasma was measured by a flame photometry; the daily and cumulative balance of these ions was calculated.

At the end of the experimental periods all the animals were sacrified for simultaneous morphological and functional studies of adrenals and kidneys.

The adrenals were incubated in Krebs-Ringer solution with glucose for the estimation of adrenocorticoid production. The extraction of hormones from the incubation medium was carried out with methylene–chloride with subsequent purification and separation of corticosteroids by means of thin-layer chromatography [13, 14].

For the studies of morpho-functional state of adrenals the diameters of nuclei and nucleoli were estimated by means of ocular micrometer on slices stained after Brachet [15]. The volumes of nuclei and nucleoli were calculated by formulas of sphere and/or ellipse, depending on the form. The width of the glomerular zone was measured on slices stained with haematoxyline–eosin with ocular-micrometer.

Identical areas of kidneys were taken for estimation of renin content and for morphological studies. Renin was determined by somewhat modified Gross' [17] technique: saline kidney extracts were incubated with EDTA-diluted horse serum. Renin content was expressed in μg of angiotensin formed (angiotensin-equivalents) per lg of kidney cortex tissue. The amount of angiotensin in a sample was estimated in a bioassay by the pressor response of nembutal–(30 mg/kg)–anaesthetized nephrectomised rats to which mechamilamine (1 mg/kg) had been given, as compared to the effect of a synthetic angiotensin standard.

For histological studies, pieces of kidneys were fixed in Zenker solution, installed in dioxan and embedded in paraffin. Besides where conventional histological techniques were applied, 2–4μ slices of kidney were stained after Bowie according to a method described by Pitcock & Hartroft[16]. The kidney JGA morpho-functional activity was estimated by the value of JGI calculated by the method of Hartroft & Hartroft[17].

RESULTS

Each experimental group of animals was compared with a corresponding control group. There was a non-significant difference in the controls, possibly due to seasonal variations and some difference in diet. As expected, administration of large doses of cortisol to dogs sharply decreased, the production of endogenous glucocorticoids, especially cortisol (Table 1). Against this background the considerable increase in aldosterone production is very remarkable (Fig. 1). The determination of *in vitro* corticosteroid production, though it does not characterise the absolute hormone secretion, is in wide use experimentally as an index of adrenal cortical functional activity[18, 19, 20]. This method provides the possibility of avoiding the stress resulting from the canulation of adrenal vein. The corticosteroid production in this group of animals was calculated taking into account the change of ratio in widths between the glomerular and fasciculo-reticular zones found in the histological study of adrenal cortex. As one can see from the data (Table 1) the size of the glomerular zone in treated animals was unchanged, and that of the fasciculo-reticular zone was sharply decreased. The extrapolation of corticosteroid production to a whole adrenal taking into account the weight of the animal enables us to take into account the selective atrophy of the fasciculo-reticular zone. Sodium and potassium excretion on the 10th day of cortisol injection in both the control and experimental animals corresponded to electrolyte intake (60–70 mEq of Na and about 30 mEq a day). The lack of changes in plasma concentration of these ions and the value of exchangeable sodium suggest that electrolyte metabolism is stable during the cortisol injection in these experiments. There were no changes in activity of the kidney renin–angiotensin apparatus as judged by the JGI value (Table 2).

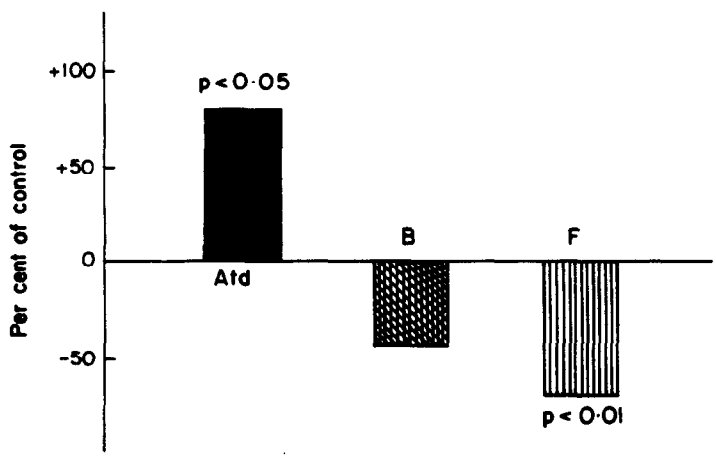

Fig. 1. Corticosteroid production changes after administration of large doses of cortisol.

Table 1. Corticosteroid production *in vitro* and morphological state of adrenal cortex after 10 days of cortisol injections (3 mg/kg/day)

Group of animals	No.	Corticosteroid production (μg/100 mg/hr)			Corticosteroid production*			Zone sizes μ		% z. glomerulosa to all cortex
		Aldosterone	Corticosterone	Cortisol	Aldosterone	Corticoster.	Cortisol	Z. glomer.	Z. fascic. + Z. retic.	
Control	8	$\pm \frac{0{\cdot}22}{0{\cdot}04}$	$\pm \frac{0{\cdot}85}{0{\cdot}19}$	$\pm \frac{0{\cdot}86}{0{\cdot}09}$	$\pm \frac{0{\cdot}20}{0{\cdot}02}$	$\pm \frac{0{\cdot}78}{0{\cdot}19}$	$\pm \frac{0{\cdot}75}{0{\cdot}13}$	$\pm \frac{116}{1{\cdot}9}$	$\pm \frac{490}{21{\cdot}6}$	19
Exper.	9	$\pm \frac{0{\cdot}45}{0{\cdot}10}$	$\pm \frac{0{\cdot}52}{0{\cdot}07}$	$\pm \frac{0{\cdot}21}{0{\cdot}06}$	$\pm \frac{0{\cdot}38}{0{\cdot}07}$	$\pm \frac{0{\cdot}44}{0{\cdot}05}$	$\pm \frac{0{\cdot}19}{0{\cdot}05}$	$\pm \frac{121}{5{\cdot}7}$	$\pm \frac{364}{15{\cdot}1}$	25
		$p < 0{\cdot}05$	N.S.	$p < 0{\cdot}01$	$p < 0{\cdot}05$	N.S.	$p < 0{\cdot}01$	N.S.	$p < 0{\cdot}01$	

Mean ± S.E.
N.S., not significant.
*Expressed in μg/g adr. wt./kg b.wt./hr.

Table 2. The state of kidney JGA and salt–water balance in dogs after 10 days of cortisol injections (3 mg/kg/day)

Group of animals	No.	JGI	Excretion of electrolytes (mEq/24 hr)		Plasma electrolyte concentration (mEq/l)		Exchangeable sodium (mEq)
			Na	K	Na	K	
Control	5	13·3 ± 0·8	63·2 ± 4·34	27·7 ± 2·19	149·2 ± 1·12	4·70 ± 0·07	865 ± 76
Experim.	5	13·6 ± 1·5	64·2 ± 2·36	29·5 ± 2·20	149·6 ± 2·00	4·71 ± 0·08	835 ± 55
		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

Mean ± S.E.
N.S., not significant.
JGI, juxtaglomerular granularity index.

Different results were obtained in the experiments where large doses of aldosterone were administred (Table 3). Exogenous aldosterone administration almost completely suppressed the endogenous aldosterone production without effecting cortisol production (Fig. 2). The width of the glomerular zone diminished but the size of nuclei and nucleoli remained the same as in controls (Table 3).

In the kidneys of treated animals a sharp decrease in JGI and renin level was found (Table 4). According to Gross [7], the kidney renin level in chronic experiments reflects the concentration of enzyme in blood plasma. Thus, the data obtained characterise to some extent the state of the renin–angiotensin system as a whole. In aldosterone treated animals the blood sodium concentration was unchanged, but that of the potassium decreased slightly (Table 4). The study of sodium dynamics revealed some interesting features. Initially there was sodium retention, but by the fifth day of exogenous hormone administration, "escape" effect took place. The urinary sodium and the exchangeable sodium then returned to initial levels. However, the cumulative sodium balance continued to be positive, showing that the retained sodium had not yet been excreted from the body (Fig. 3).

DISCUSSION

The experiments showed that injection of cortisol in dose of 3 mg/kg did not change the metabolism of sodium significantly. Possibly, this is the cause of the

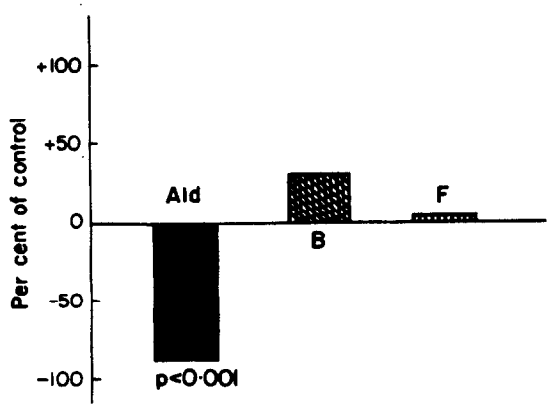

Fig. 2. Corticosteroid production changes after administration of large doses of aldosterone.

Table 3. Corticosteroid production *in vitro* and morphological state of adrenals in dogs after 5 days of aldosterone injections (100 μg/kg/day)

Group of animals	No.	Corticosteroid production (μg/100 mg/hr)			Zona glomerulosa			Size of z. reticulata plus z. fasciculata
		Aldosterone	Corticosterone	Cortisol	Size	Nuclear vol.	Nucleolar vol.	
Control	6	0·15 ± 0·03	0·33 ± 0·03	0·46 ± 0·10	100·4 ± 1·1	49 ± 0·9	0·7 ± 0·04	466·8 ± 23·4
Experim.	5	0·02 ± 0·02	0·43 ± 0·05	0·47 ± 0·03	68·5 ± 0·9	50 ± 0·8	0·8 ± 0·05	457·1 ± 22·1
		$p < 0·01$	N.S.	N.S.	$p < 0·01$	N.S.	N.S.	N.S.

Mean ± S.E.
N.S., not significant.

Table 4. The state of kidney JGA and salt–water balance in dogs after 5 days of aldosterone injections (100 μg/kg/day)

Group of animals	No.	JGI size	Renin in kidney μg/g-angio Eq	Electrolyte excretion (mEq/24 hr)		Plasma electrolyte concentr. (mEq/l)		Exchangeable sodium (mEq/kg)
				Na	K	Na	K	
Control	9	13·3 ± 0·8	3·6 ± 0·8	69 ± 4	18·1 ± 1	154 ± 1	4·5 ± 0·1	711 ± 27
Experim.	5	4·6 ± 0·9	0·9 ± 0·2	69 ± 5	26·0 ± 2	153 ± 1	4·1 ± 0·1	704 ± 29
		$p < 0·01$	$p < 0·01$	N.S.	$p < 0·01$	N.S.	$p < 0·05$	N.S.

Mean ± S.E.
N.S., not significant.

lack of change in the state of the renin–angiotensin system, as judged by the JGI magnitude. It follows from this, that a relatively high aldosterone production seen in the experiments is due not to the renin–angiotensin system activation but to an action of other factors.

In the discussion of the mechanisms resulting in the functional changes of the adrenal cortex under the action of cortisol; the two pathways of the feedback system must be remembered, one of which involving the hypothalamus-pituitary [1, 2], the other – hormone-producing adrenal tissue [21, 22, 23]. The depressed secretion of ACTH which normally stimulates the protein synthesis in adrenals [24] and the direct inhibitory effect of exogenous cortisol on steroidogenesis lead to atrophy of the fascicular zone of the adrenal cortex with a preferential suppression of cortisol production. Corticosterone production could have been maintained to a certain extent because of the intact glomerular zone. The absence of any visible morphological changes in the glomerular zone after administration of cortisol has been described [25]. Moreover, under the influence of cortisol, in the glomerular zone of adrenals there is increased 3β-hydroxysteroid dehydrogenase activity [26]. Cortisol administration brings about an induction of glyconeogenetic enzymes and an increase in tissue acetyl-Co A content [27]. In this way cortisol, acting through a feedback mechanism upon pituitary and the fascicular zone, creates at the same time a favourable situation for an enhancement of glomerular zone function.

The data presented here are somewhat at variance with the work of Newton & Laragh [28] on men, which showed the opposite interaction of aldosterone excretion in healthy subjects with a controlled sodium intake. We also observed that cortisol did not influence the salt–water balance or the renin–angiotensin system.

Possibly, under these conditions the aldosterone production is stimulated by the "hypothetical pituitary hormone" the secretion of which is not blocked by cortisol. A rationale for such a hypothesis was found in the data on the permissive effect of the "pituitary hormone" upon the aldosterone secretion during sodium deficiency [29]. A no less plausible hypothesis would be that the endogenous angiotensin concentration proves to be more efficient under the given conditions [30].

At first glance, this hypothesis seems to contradict the data of Gahong *et al.* [31] that in dogs treated with high doses of corticosteroid there is an adequate aldosterone response to the stimulating effect of exogenous angiotensin II. But one should remember that Ganong *et al.* [25] used a synthetic corticosteroid, depomedrol, injected for a month which decreased the adrenal weight considerably, as in hypophysectomy. Perhaps the difference in effect depends not only on the duration of the drug administration but also on its chemical properties. Depomedrol probably acts upon the adrenal tissue in a somewhat different way to cortisol. According to Farrell *et al.* [32] administration of high doses of cortisol and cortisone to dogs for 5 weeks decreases ACTH content of the pituitary by 20% and does not decrease aldosterone secretion rate in the treated animals. On some days the aldosterone secretion rate was even higher than in the controls.

The results of our studies show an opposite effect obtained in experiments with administration of high doses of exogenous aldosterone. A normal level of cortisol production reflected the absence of any changes in ACTH secretion. At the same time, in the treated animals there was a sharp decrease in kidney juxtaglomerular granularity index and renin level.

It is logical to suppose that a decrease in aldosterone production by adrenals depends on the decrease of renin level. But the question arises, why ACTH and other "Pituitary hormones" cannot maintain aldosterone production at the normal level under renin deficiency. As Ganong *et al.*[33] suppose, a long-term existence of an adrenal under the conditions of a low level of circulating renin leads to a decrease in adrenocortical sensitivity to aldosterone-stimulating action of ACTH and angiotensin II. The mechanism remains obscure.

In our experiments, it is remarkable that aldosterone-induced decrease in kidney renin level is accompanied by a selective inhibition of aldosterone production. As seen in Fig. 2, the production of the basic aldosterone precursor, corticosterone, is not only undiminished but has even some tendency to increase. It has been established that sodium deficiency stimulates the transformation of corticosterone into aldosterone, in dogs[34] and sodium excess inhibits the formation of aldosterone, enhancing the transformation of 18-hydroxycorticosterone into 18-hydroxy-11-dehydroxycorticosterone[35]. The hypothesis of a direct action of aldosterone through a feedback mechanism on the 18-hydroxylation cannot be supported, as it is known that, unlike cortisol, aldosterone does not affect corticosteroid production when acting directly on adrenal gland[4, 36]. Possibly, the effect of aldosterone on the adrenals through a feedback mechanisms is mediated through electrolyte metabolism.

We have no data as to sodium concentration in the glomerular zone cells of treated animals. The hypothesis put forward is based upon the comparison of dynamics of cumulative sodium balance and the total exchangeable sodium of the body. As seen in Fig. 3, by the end of the period of aldosterone administration there was a considerable gap between the positive cumulative sodium balance level and the total exchangeable sodium level which had returned to the initial value. The discrepancy between these indices of sodium metabolism could be interpreted as indicating that a considerable part of the sodium retained in the body is in a non-exchangeable form, inaccessible to radioactive label. Possibly,

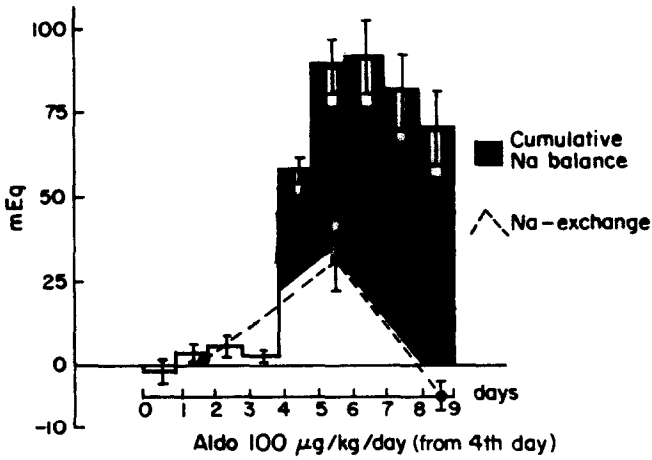

Fig. 3. Cumulative sodium balance and exchangeable sodium changes during aldosterone administration in dogs. ΔNa-exchang. – exchangeable sodium in mEq (difference from initial level) – mean ± SEM (by pair method).

some of the retained sodium also accumulates in structures of the glomerular zone. The accumulation of sodium in the glomerular zone cells could inhibit the transformation of corticosterone into aldosterone.

Some insight into the intracellular processes going on in parallel with the above described was obtained by the estimation of the size of nuclei and nucleoli and width of glomerulosa. It is known that the volume of nucleolus is closely connected with ribosomal RNA and protein synthesis in ribosomes[37]. During the increase of aldosterone secretion due to sodium deficiency the nucleolar volume in mice increases[38]. In our previous experiments with inferior vena cava constriction in dogs the enhancement of renin and aldosterone production was also followed by an increase in nuclear and nucleolar volume[39].

After the injection of aldosterone into normal animals the renin and aldosterone production and width of *z. glomerulosa* are sharply decreased at the same time, in the glomerular zone cells the size of nuclei and nucleoli being the same as in control. The latter suggests a normal synthesis of ribosomal proteins[37].

The decrease of glomerulosa width with no change in nuclear and nucleolar volume, suggest the selective inhibition of the process in some cytoplasmatic structures, possibly in endoplasmatic reticulum, mitochondria or microsomes, which are in close relation with corticosteroid synthesis[40, 41]. There is evidence[42] that the transformation of corticosterone into aldosterone in dogs (but not in rats) does not depend on protein synthesis at ribosomal level. It is notable that aldosterone does not affect protein synthesis in rat adrenals[43].

On this basis we suggest that the feedback mechanism in the action of aldosterone may be mediated through the increase of sodium concentration in glomerular zone cells and that it does not involve the ribosomal apparatus which is responsible for earlier stages of corticosteroid biosynthesis. The peripheral feedback mechanism mediated through the sodium has some advantage, as with this the transformation of corticosterone into aldosterone is selectively blocked and thus the other pathways of steroidogenesis are preserved. The considerable time required for the realisation of this effect may be connected with the slow rate of the accumulation of unexchangeable sodium in tissue structures. Two feedback mechanisms in the regulation of aldosterone secretion can be distinguished one of which closes at adrenal level and the other at the level of kidney through renin–angiotensin apparatus.

The present data provides evidence for the existence of a similarity in the behaviour of pituitary-adrenal and renal–adrenal regulation systems consisting of direct reflex and of feedback relations. Interaction of these systems at the adrenal level maintains an adequate secretion of corticosteroid hormones in keeping with the needs of the organism.

REFERENCES

1. F. E. Yates and J. Urquhart: *Physical Rev.* **42** (1962) 359.
2. E. Endröczi, K. Lissak and M. Tekers: *Acta physiol. hung.* **18** (1961) 281.
3. E. C. Biglieri and W. F. Ganong: *Excerpta Med.* **51** (1962) 223.
4. J. Blair-West. J. P. Coghlan. D. A. Denton. J. R. Goding. M. Wintour and R. D. Wright: *Endocrinology* **77** (1965) 501.
5. J. Blair-West. G. P. Coghlan and D. A. Denton: *Acta endocr.. Kbh* **41** (1962) 61.
6. L. Tobian: *Physiol. Rev.* **40** (1960) 280.
7. F. Gross, H. Beunner and M. Ziegler: *Rec. Progr. Hormone Res.* **21** (1965) 119.
8. J. W. Conn: *JAMA* **190** (1964) 213.
9. J. de Champlain, J. Genest, R. Veyratt and R. Boucher: *Archs intern. Med.* **117** (1966) 355.

10. E. A. Eilers and R. E. Peterson: *Prog. 46th Mtg. Endocrinol. Soc.*, p. 62, Oklahoma City (1964).
11. A. C. Barger, G. M. Wilson, H. Z. Price, R. S. Ross, Z. Broodes and E. A. Bolings: *Am. J. Physiol.* **180** (1955) 387.
12. I. Sh. Sterental, G. S. Chudnovsky: In *Corticosteroid Regulation of Salt–Water Homeostasis* (Edited by M. G. Kolpakov), p. 223. "Nauka", Novossibirsk (1967).
13. M. G. Kolpakov, P. M. Krass, R. A. Samsonenko and G. S. Chudnovsky: *Proc. Acad. Sci. USSR*, **172** (1967) 233.
14. T. I. Ivanenko, R. N. Shchedrina and M. I. Kondror: *Probl. endocr.* **14** (1968) 72.
15. J. Brachet: *C. R. Soc. biol., Paris* **133** (1940) 88.
16. J. A. Pitcock and P. M. Hartroft: *Am. J. Pathol.* **34** (1959) 863.
17. P. M. Hartroft and W. S. Hartroft: *J. exp. Med.* **97** (1953) 415.
18. I. Van der Vies: *Acta endocr.* **33** (1960) 59.
19. B. Van der Wal and D. de Wied: *Acta Physiol. Pharmacol. Neerl.* **13** (1964) 110.
20. G. P. Vinson and J. C. Rankin: *J. endocr.* **33** (1965) 195.
21. F. G. Peron, F. Moncloa and R. J. Dorfman: *Endocrinology* **67** (1960) 379.
22. W. C. Black, R. S. Crampton, A. S. Verdesca, A. S. Nedeljkovic and J. G. Hilton: *Am. J. Physiol.* **201** (1961) 1057.
23. M. G. Kolapkov, G. S. Shishkin, S. G. Kolaeva and N. D. Lutsenko: *Probl. endocr.* **13** (1967) 60.
24. W. W. Davis and L. D. Garren: *J. biol. Chem.* **243** (1968) 5153.
25. O'Donnell W., St. S. Fajans and J. Weinbaum: *Archs intern. Med.* **88** (1951) 28.
26. C. Cavallero and G. Chiappino: *Experientia* **19** (1963) 569.
27. W. Senbert: In *18 Colloq. Ges. Physiol. Chem.*, p. 192. Mosbach/Baden. Berlin, Heidelberg, New York (1967).
28. M. A. Newton and J. H. Laragh: *J. Clin. endocr.* **28** (1968) 1014.
29. W. P. Palmore and P. J. Mulrow: *Science* **158** (1967) 1482.
30. M. G. Kolpakov: *Proc. Acad. Sci. USSR* **185** (1969) 475.
31. W. F. Ganong, D. L. Pemberton and E. van Brunt: *Endocrinology* **81** (1967) 1147.
32. G. Farrell, R. Banks and S. Koletsky: *Endocrinology* **58** (1956) 104.
33. W. F. Ganong, E. Y. Bigliery and P. J. Mulrow: *Rec. Progr. Hormone Res.* **22** (1966) 381.
34. W. W. Davis, L. R. Burwell, G. Kelley, A. F. T. Casper and F. C. Bartter: *Biochem. biophys. Res. Commun.* **22** (1966) 218.
35. D. C. Sharma: In *Rec. Prog. Hormone Res.* **22** (1966) 381.
36. C. Laplante and J. Stachenko: *Can. J. Biochem.* **44** (1966) 85.
37. J. Sirlin and J. Jacob: *Nature, Lond.* **195** (1962) 114.
38. B. Miller: *Metabolism* **12** (1963).
39. S. G. Kolaeva: In *Aldosterone and Adaptation to Salt–Water Regime Changes*, p. 38. "Nauka", Novossibirsk (1968).
40. F. Gvacomell, J. Wiener and D. Spire: *J. cell Biol.* **26** (1965) 39.
41. M. Nishikawo, L. Marone and T. Sato: *Endocrinology* **72** (1963) 197.
42. W. W. Davis, L. R. Burwell, A. J. T. Casper and F. C. Bartter: *J. clin. Invest.* **47** (1968) 1425.
43. L. B. Morrow, P. J. Mulrow and G. N. Borrow: *Endocrinology* **80** (1967) 883.