ISSN 0006-2979, Biochemistry (Moscow), 2010, Vol. 75, No. 3, pp. 353-366. © Pleiades Publishing, Ltd., 2010.
Published in Russian in Biokhimiya, 2010, Vol. 75, No. 3, pp. 431-446.

Blood Content of Tyrosine Is an Index of Glucocorticoid Action on Metabolism

I. T. Rass

Center of Theoretical Problems of Physicochemical Pharmacology, Russian Academy of Sciences, ul. Kosygina 4, 119991 Moscow, Russia; E-mail: irma2@kmail.ru

Received November 12, 2008
Revision received June 15, 2009

Abstract—Glucocorticoid hormones directly or indirectly control virtually all metabolic and physiological processes. Glucocorticoids are also shown to act on a multitude of genes, enzyme systems, and proinflammatory factors, but for these hormones there is no representative index of action on metabolism similar to glucose content in blood for insulin. The absence of such an index prevents the assessment of tissue provision with these hormones under various conditions and seems to be an essential cause of complications associated with the clinical use of glucocorticoid preparations. Considering specific features of tyrosine metabolism and data obtained experimentally and on a clinical model (adrenalectomy in rats and substitution therapy in endocrine disease), blood content of this amino acid seems promising as such an index. Based on comparing results of glucocorticoid treatment in patients with systemic lupus erythematosus with changes in their blood tyrosine contents, the pharmacological effect of glucocorticoid preparations is suggested to be mainly due to compensating a relative shortage of these hormones.

DOI: 10.1134/S0006297910030120

Key words: glucocorticoids, hormonal provision of tissues, blood tyrosine, tyrosine aminotransferase

Preparations of glucocorticoid (GC) hormones and their synthetic analogs (prednisolone, dexamethasone, triamcinolone, methylprednisolone, etc.) are widely used in clinical medicine mainly as the most powerful anti-inflammatory drugs also possessing anti-allergic, immunosuppressive, antitoxic, and anti-shock actions. However, use of these unique preparations is hampered by impossibility of predicting their effects in special cases and by frequent complications sometimes comparable in severity with the disease itself.

Glucocorticoid preparations act on all manifestations of the inflammatory and immune reaction and as analogs of natural hormones inevitably act also on metabolic and physiological processes. And it seems that just the variety of effects of GCs has prevented the finding of a specific representative index of their action on the organism that would allow the determination of the true need of a patient for these preparations, forecast their efficiency, and control their dose. The absence of such an index makes very difficult an individual approach for the treatment of patients – all depends on the physician's intuition and experience.

In the 1970s the author proposed to use as such index blood content of tyrosine, which is mainly determined by activity of a GC-dependent enzyme hepatic tyrosine aminotransferase (TAT), i.e. to profit from a result of GC action on metabolism.

60 YEARS OF USING GLUCOCORTICOIDS

Glucocorticoid therapy was born September 21, 1948, in the Mayo Clinic: Doctor P. Hench injected cortisone isolated by E. Kendall from the adrenal cortex to a patient with rheumatoid arthritis, and after three injections the nearly motionless patient could rise up and go [1]. In July 1949 the report by P. Hench about results of using cortisone in various diseases created a sensation in the International Rheumatological Congress in New York. And GCs literally streamed into clinical medicine. Results of their use were so impressive that already in 1950 P. Hench, E. Kendall, and T. Reichstein were awarded the Nobel Prize in Physiology and Medicine for

Abbreviations: ACTH, adrenocorticotropic hormone; AGS, adrenogenital syndrome; GC, glucocorticoid; HHACS, hypothalamus-hypophysis-adrenal cortex system; *p*OPP, *p*-oxyphenylpyruvic acid; PS, prednisolone; SLE, systemic lupus erythematosus; TAT, tyrosine aminotransferase.

studies on adrenal cortex hormones, determination of their structure and biological action.

During some successive years various derivatives of cortisone and hydrocortisone were synthesized, and the 1950s were a period of extremely extensive spontaneous clinical testing of new preparations. Preparations of GCs occurred to be the only drugs that combine the powerful anti-inflammatory effect with immunosuppressive, anti-allergic, and antitoxic effects; physicians prescribed GC preparations as the most efficient drugs in rheumatism, collagenoses, rheumatoid arthritis, bronchial asthma, severe dermatoses, hemolytic anemia, acute lympho- and myeloleukemia, etc. In the virtually lethal systemic lupus erythematosus (SLE), GCs saved the lives of many patients.

However, the initial euphoria was rather rapidly replaced by confusion and then by disappointment. Serious side effects predicted and described by P. Hench himself [2] were observed in many patients taking the hormonal preparations [3-5]. These are hypercorticism presenting the clinical picture of Cushing's syndrome including disorders in mineral metabolism, hypertension and as a result an increased frequency of cardiovascular diseases, secondary infections, steroid diabetes, osteoporosis, and aseptic osteonecrosis resulting in invalidity; in some patients gastrointestinal ulcers and hemorragias appeared. A prolonged taking of GC preparations induced adrenocortical failure because of disorders in regulation of the hypothalamus–hypophysis–adrenal cortex system (HHACS). In some cases this secondary adrenocortical failure made it impossible to abolish GC administration because of development of the so-called withdrawal syndrome – exacerbation of the disease – and could be the cause of the patients' death in occasional stress situations. Surprisingly, the therapeutic effect of GC preparations did not depend on the content of the endogenous GC hormones and was absent in some patients with the same disease. Use of GC preparations was accompanied by changes in various clinical and laboratory parameters, but these changes were not specific and did not allow clinicians either to predict the effect or control the dose of GCs [6, 7].

Because of absence of objective laboratory indications of the demand for GCs, clinical indications are revised and defined more accurately during 60 years: an exact diagnosis and clear pathomorphologic symptoms are required. Up to now empirical schemes of the hormonal treatment of different diseases are reproduced in various guidebooks, reference books, and special papers without essential changes [8-11]. Pulse-therapy, chronotherapy, alternate-day therapy, as well as well-timed medicamentous prevention and correction of side effects are useful for diminishing their frequency and severity but fail to solve the problem.

Appearance of hypercorticism signs is usually considered a signal for lowering the dose of GC preparations, and the regimen of the decrease and withdrawal of these drugs is given special attention [12, 13].

GC preparations are used very widely: about a million patients obtain them permanently only in the USA [14], and no less than 10 million new prescriptions are made every year [15], thus, side effects are still a serious problem [16-18].

However, signs of medicamentous hypercorticism and the secondary adrenocortical failure really are not "side effects", because they are not caused by the toxicity of the preparation but are manifestations of an increased action of GCs on metabolism or the result of disturbance of interhormonal interaction, i.e. they are caused just by the hormonal features of GC preparations.

Therefore, from the very beginning of the "glucocorticoid era" chemists and pharmacologists had to separate therapeutic effect of GC preparations from undesirable manifestations of their hormonal features, in particular, the effect on the carbohydrate metabolism and suppression of HHACS. Some success has been achieved. Thus, cortisol fluoro-derivatives dexamethasone and betamethasone do not cause sodium retention and their clinical efficiency is 25-30 times higher than that of their natural prototype, but the adrenal cortex suppression is more severe and prolonged.

At present, there are various GC preparations for different purpose, and the list of such preparations is constantly increasing. GCs are frequently used also in combination with antibiotics, antiviral, or anti-thrombotic agents.

On the basis of "classical" GC preparations new ones are also synthesized: lazaroids, or 21-aminosteroids, which possess antioxidant properties and are promising as neuroprotectors [19]; nitrosteroids with a labile nitro group in the steroid nucleus [20] and an increased anti-inflammatory activity mediated by nitration of GC receptors. Liposomal forms of GCs [21] ensure a high local concentration of the preparation, which is important at topical application and in the treatment of some tumors.

Mechanisms of the anti-inflammatory action of GCs have been intensively studied during all 60 years of their use. The anti-inflammatory action of GCs was shown to be associated with an increase in expression of genes encoding anti-inflammatory proteins and with suppression of transcription of genes responsible for generation and release of inflammation mediators. GCs were also shown to induce apoptosis of immunocompetent cells [22-25].

Mechanisms of undesirable effects of GCs are not quite clear, but some of them were shown to be realized after the binding of GCs with their receptors. This observation has created a fundamental possibility for separating anti-inflammatory and side effects of GC preparations [26-29] and determined the direction for searches and synthesis of new steroidal and non-steroidal ligands – Selective Glucocorticoid Receptor Agonist (SEGRA),

which would be bound with GC receptors and decrease the frequency of side effects [15, 30-33].

Nevertheless, hormonal therapy still remains a field of clinical empiricism. As formerly, it is unclear why GC hormones – the organism's natural products or their synthetic analogs – are the most efficient anti-inflammatory agents and there is no objective index of a patient's need for these hormones. Up to now it is recommended to use GCs in "doses required to control the disease but the most lowest to minimize side effects" [34]. However, there is a possibility to assess the individual's need for GC preparations using a manifestation of their hormonal features – the regulation of tyrosine content in blood, which is determined by the activity of a GC-dependent enzyme hepatic tyrosine aminotransferase (TAT).

The idea of using the blood tyrosine content as a promising representative index of GC action on metabolism and characteristics of the tissue provision with these hormones and/or the hormonal preparations was proposed in the work [35] proceeding from the author's observations on patients with SLE.

GLUCOCORTICOIDS ARE HORMONES OF HOMEOSTASIS AND STRESS

Prescribing GCs to his patients, Dr. Hench took into account his own observations that conditions of patients with rheumatoid arthritis improved in pregnancy and in jaundice and also data of physiologists, first of all Hans Selye, on the increase in the adrenal cortex activity under stress conditions [36, 37], and searched for a corresponding natural product of the organism.

In its turn, the clinical use of GC preparations stimulated studies on HHACS functions and action mechanisms of the adrenal cortex hormones, but attention was mainly given to pharmacological effects of synthetic GCs and the physiology of the natural hormones was a little neglected.

Adrenals are vitally important organs. Extirpation of adrenals in humans and the majority of animals leads to general disorders in metabolism, increasing weakness, hypoglycemia, fall of arterial pressure, anorexia, diarrhea, and, as a rule, death on the 4-5th day. Injection of GCs resulted in a rapid improvement of condition and prevented the post-adrenalectomy death [38]; however, injection of GCs did not increase the working capacity of healthy animals and humans [39, 40].

Glucocorticoid hormones (cortisol in humans and corticosterone in rodents) do not have a specific target organ; comparable concentrations of their receptors are found in virtually all tissues and organs [41], and this ensures an extremely broad range of metabolic and physiological effects of these hormones. GCs are necessary for normal metabolism of carbohydrates, proteins, lipids, water, and electrolytes, for supporting vascular tonus, functioning of cell membranes and subcellular structures, and also for providing activities of other hormones. Really, GCs are total action hormones and, thus, play a very essential, if not the decisive, role in supporting homeostasis [42].

The increased secretion of GCs by the adrenal cortex underlies Cushing's syndrome [43]. The high incidence of the type II diabetes and obesity is now thought to be associated with overproduction of GCs [44]. A decreased adrenocortical activity is revealed in many patients with chronic allergy, chronic fatigue syndrome, and autoimmune disorders [45], and stress can provoke exacerbation of such states. Insufficient activity of HHACS can be a prerequisite for development of rheumatoid arthritis [46, 47] and bronchial asthma [48].

Suppression of the thymico-lymphatic apparatus under stress conditions was observed by Selye. GCs inhibit functions of the majority of immunocompetent cells [49], and pharmacological doses of GCs are used as immunosuppressors; however, GC hormones are required for supporting immune homeostasis [50, 51]. Gastrointestinal tract ulcers under stress conditions described in some old works [52, 53] are now believed to be caused not by an increased secretion of GCs but by microcirculation disorders in the stomach walls and action of the acid [54]. In the works [55, 56] GCs are shown to protect the gastric mucosa in the presence of some ulcerogenic agents or under conditions of severe stress.

In addition to supporting homeostasis under physiologically normal conditions, HHACS plays a decisive role under stress conditions. Under shock of different genesis, in contests, *status asthmaticus*, etc. blood content of GCs can become tenfold higher than their normal content at rest [57]. Cortisol secretion by the adrenal cortex is increased also in infectious diseases caused by different agents: bacteria, rickettsia, or viruses [58]. The cortisol content usually increases in a patient's blood on the day of surgery, and sometimes the absence of such an increase is associated with collapse [59]; therefore, in some cases GC coverage is needed [60].

The increased secretion of GCs under stress conditions is a classical endocrine reaction, although it is not clear whether this reaction supplies an increased energy demand of the organism in stress or moderates excessive local inflammations [61-63]. But conservation of the increased content of GCs for a long time can lead to pathology.

But undoubtedly there is a certain optimum of the adrenal cortex activity and GC provision in a disease (or in stress situation). Thus, upon infection of adrenalectomized mice and rats, respectively, with virulent *Pneumococcus* and *Trypanosoma lewisi*, the optimal survival was recorded in animals which were injected daily with hydrocortisone in the dose corresponding to adrenocortical reaction of un-operated infected animals [64,

65]. In mice infected with *Pneumocystis carinii* suspension the best survival was recorded on the injection of 1 mg cortisone, whereas higher doses led to development of bacterial superinfection [66]. The existence of such an optimum is also confirmed by the above-mentioned recommendation to use the "minimal effective doses" of GC preparations.

However, to find this optimum under different conditions is rather difficult. To characterize the adrenal cortex activity, it is necessary to determine blood contents of free (biologically available) cortisol and of transcortin, to assess adrenal cortex reserves with some functional tests, to determine GC metabolites in urine, etc. But because of pronounced individual differences (cortisol content in blood of healthy humans at 6.00-8.00 a.m. can vary from 6 to 23 μg/100 ml, and the response to functional tests can differ tenfold), such an examination cannot reveal whether the hormonal provision of tissues corresponds to a subject's needs in a given situation. Moreover, there is no sense in this examination in patients taking GC preparations.

HEPATIC TYROSINE AMINOTRANSFERASE AND TYROSINE METABOLISM

Injection into rats of cortisone or hydrocortisone induces synthesis of two hepatic enzymes: tryptophan dioxygenase (EC 1.13.11.11) and tyrosine aminotransferase (TAT; EC 2.6.1.5), which catalyze, respectively, the first stages of tryptophan and tyrosine oxidation [67, 68]. The influence of GCs was especially pronounced in adrenalectomized animals: activities of both enzymes increased 7-10-fold 4-5 h after the injection of GCs and decreased to the basal level in 18-20 h. Synthesis of these enzymes could be also induced by injection of the corresponding substrates, but for inducing TAT synthesis GCs were required, endogenous, or injected into adrenalectomized animals. The activity of TAT could also be induced by injecting other hormones and some substances but it was always mediated through GCs [69, 70].

GCs control synthesis of various enzymes [71, 72], but synthesis of the hepatic TAT depends on them most clearly, and in a certain range of GC concentrations the activity of the *de novo* synthesized TAT in rat liver is proportional to amount of the injected GC preparation [73, 74].

The binding of different GC preparations with specific GC receptors correlates with TAT synthesis in rat hepatoma culture and in rat liver *in vivo* [75, 76] and with manifestations of some hormonal features: death of lymphocytes in lymphoma culture, a decrease in glucose oxidation in tissues, inhibition of amino acid transport across the hepatocyte membrane [77, 78]. Activities of human and animal hepatic TATs depend on GCs similarly [79]; therefore, TAT synthesis in cell culture or in the liver of adrenalectomized rats can be used to characterize biological effects of GC preparations [80]. GCs are also shown to activate transcription of the hepatic TAT gene [81].

Due to these specific features, hepatic TAT is used in studies on mechanisms of GC action and activation of GC receptors [82, 83], as an etalon system in studies on effects of GCs and proinflammatory cytokines [84], and as a model for comparing effects of hormonal preparations and endogenous corticosterone [85]. Note also that activation of hepatic TAT is used for assessment of the *in vivo* efficiency of new GC preparations: a combined multipurpose preparation [86], the non-steroidal selective agonist of GC receptors ZK216348 [30], and an N-arylpyrazolo[3,2-c]-based ligand [87].

Nevertheless, using the hepatic TAT activity as an index of GC action on metabolism on the organism level and in medical practice seems impossible.

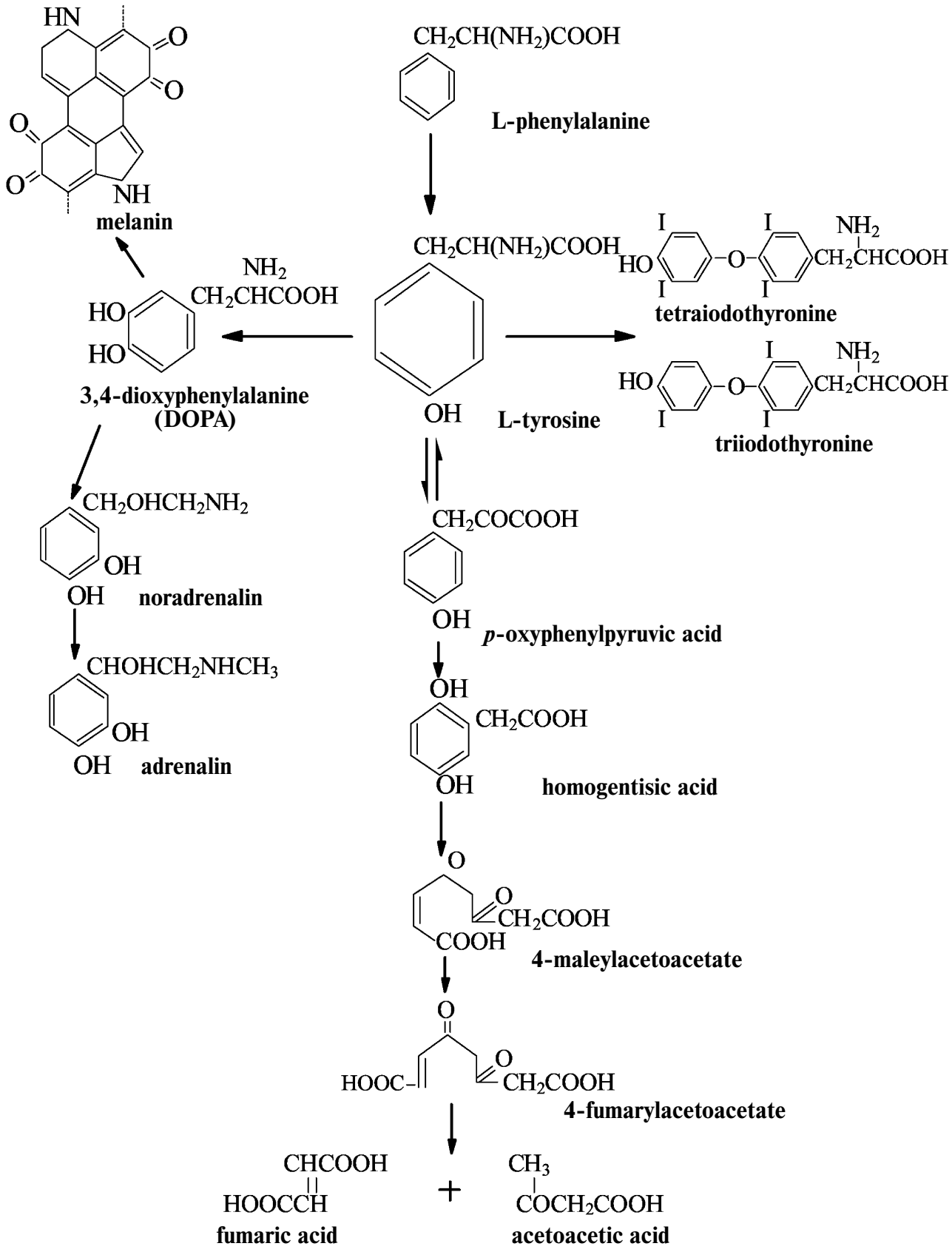
First, TAT is a tissue enzyme, and its activity can be reliably determined only in liver biopsy. Second, activity of the GC-induced TAT will be proportional to the amount of GCs entering the liver, thus, determination of the TAT activity will be equivalent to determination of GC content in blood and will fail to add anything essentially new to the routine assessment of functional activity of the adrenal cortex. And the problem of the hormonal provision of GC-dependent metabolic processes will remain unsolved.

To characterize the influence of GCs on metabolism and to assess the organism's provision with these hormones and the need in GC preparations, it is reasonable to determine not the activity of GC-dependent enzymes, but the result of their activities – just similarly to assessment of insulin action not by activities of the glycolysis enzymes but by blood glucose content. And some features of metabolism of tyrosine, which is a specific substrate of TAT, allow us to consider blood content of this amino acid as a possible representative index of GC action.

Tyrosine is produced in the organism by hydrolysis of food protein. About 30% of this amino acid is used for synthesis of catecholamines, melanin, and thyroid hormones, a portion is used for renewal of tissue proteins, and more than 60% is oxidized by the major pathway [88, 89]. The first reaction on the major oxidation pathway is transamination of tyrosine with α-ketoglutaric acid under the influence of TAT with production of *p*-oxyphenylpyruvic acid (*p*OPP). Then *p*OPP is oxidized with involvement of the appropriate oxidase in the presence of ascorbic acid [90] with production of 2,5-dioxyphenylacetic, or homogentisic, acid. The terminal products of the major pathway of tyrosine oxidation are acetoacetic and fumaric acids (Scheme).

All enzymes of the major pathway of tyrosine oxidation are most active in the liver, and TAT is the crucial enzyme.

Due to substrate induction, even on tenfold increase in protein amount in the diet the blood content of tyro-



Scheme of tyrosine catabolism (compiled after [91])

sine increases no more than by 50% above the basal level [92], and circadian variations are in the limits of ±25% [93]. Injection of GC preparations to healthy humans and animals causes a 20-40% decrease in the blood tyrosine content, and this dose-dependent decrease is the most pronounced in 4-5 h, i.e. at the maximum of the GC-induced TAT synthesis [79, 94]. A decrease in the blood tyrosine content was also recorded under stress conditions [95], presumably due to an increased generation of GCs.

Tyrosine content in blood can be increased on a decrease in the rate of its transamination caused by accumulation of *p*OPP. Thus, disorders in *p*OPP elimination results in a stable hypertyrosinemia (up to 100 μg/ml) in tyrosinosis, the hereditary disease associated with the genetically determined insufficiency of *p*OPP oxidase [96, 97]. A stable extreme hypertyrosinemia (up to 600 μg/ml) is observed in Richner–Hanhart syndrome caused by inborn TAT insufficiency [98, 99]. The incidences of these diseases in the world are 1 : 100,000 and 1 : 250,000, respectively.

Except in these very rare situations, under real conditions an increase in blood content of tyrosine can be determined by two factors: (i) liver functional inferiority leading to disturbed reception of GCs and inability of hepatocytes to synthesize adaptive enzymes including TAT, and (ii) insufficient entrance of GCs into the liver.

As a result, in the absence of extreme fluctuations in the diet tyrosine content in blood of healthy humans and animals under conditions of physiological rest is approximately the same, does not depend on sex and age, and can vary from 5.5 to 22 μg/ml in different subjects [79, 100-102]. Note that repeated determinations in the same donor gave the same result during four years [103].

In many patients with infectious hepatitis, chronic hepatitis, and liver cirrhosis tyrosine content in blood is two-threefold increased [104-106]. In some diseases, e.g. thyrotoxicosis and diabetes mellitus [107], hypertyrosinemia can be caused by secondary damage of the liver. Transient hypertyrosinemia during high-protein diet described on shortage of ascorbic acid [108] is associated with accumulation of *p*OPP as a result of disorders in its oxidation. In experiment injection of hepatotoxins caused an increase in blood tyrosine content, and this increase was higher the more pronounced was the liver necrosis [109].

In the late 1950s a group of Japanese physicians found increased contents of tyrosine in blood and urine of patients with collagenoses and supposed that disorders in metabolism of this amino acid could constitute a biochemical basis for these diseases [110, 111]. These reports stimulated intensive studies on tyrosine metabolism in collagenoses and rheumatism [112-114]. However, an increased content of tyrosine in blood and its increased excretion with urine were also found in other diseases, e.g. acute leukemia, pneumonia, acute peritonitis, etc. [115-117]. And in most cases these patients had no disorders in liver functions. The hypothesis about the role of tyrosine metabolism disorders in pathogenesis of rheumatism was not confirmed, but it was observed that the tyrosine content in blood of patients with rheumatism decreased to normal values on successful hormonal and medicamentous therapy; moreover, withdrawal of GC preparations in some cases was accompanied by an increase in the tyrosine content [100, 118]. In patients with hypocorticism withdrawal of substitution hydrocortisone injections resulted on the next day in a 25-30% increase in the blood tyrosine content, whereas contents of other amino acids were virtually unchanged [119].

BLOOD TYROSINE AS AN INDEX OF GLUCOCORTICOID ACTION AND OF TISSUE PROVISION WITH THE HORMONES

The possibility of blood tyrosine content indicating the organism's provision with GCs was studied experimentally on bilaterally adrenalectomized rats [120, 121].

According to the literature data, corticosterone content in intact rats was 19.0 ± 1.4 μg/100 ml, was undeterminable on the 4th day after adrenalectomy, and became 5.9 μg/100 ml on the 7th day [122], presumably due to corticosterone synthesis in the brown fat tissue [123] activated by excess of adrenocorticotropic hormone (ACTH) [124].

In our experiments the initial tyrosine content in blood of the rats (n = 25) was 15.0 ± 1.4 μg/ml. It increased after adrenalectomy and reached maximum on the 5th day at the most pronounced decrease in the body weight and death of 15-20% of the animals in different series of the experiment. Apparently, this picture corre-

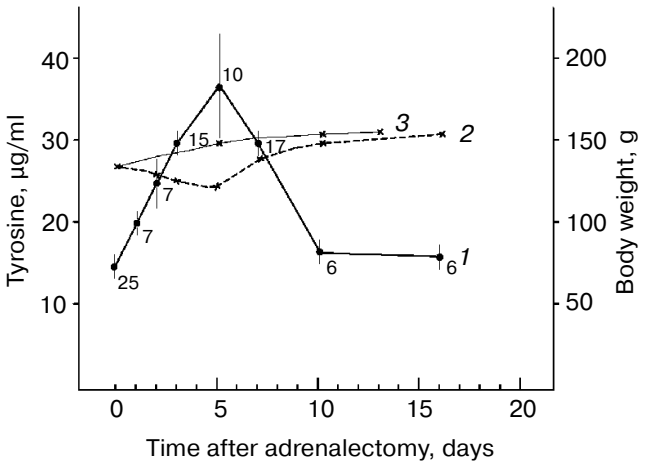


Fig. 1. Changes in blood tyrosine content (*1*) and in body weight (2) of rats after adrenalectomy; *3*) body weight of control animals. The number of animals is given by each point.

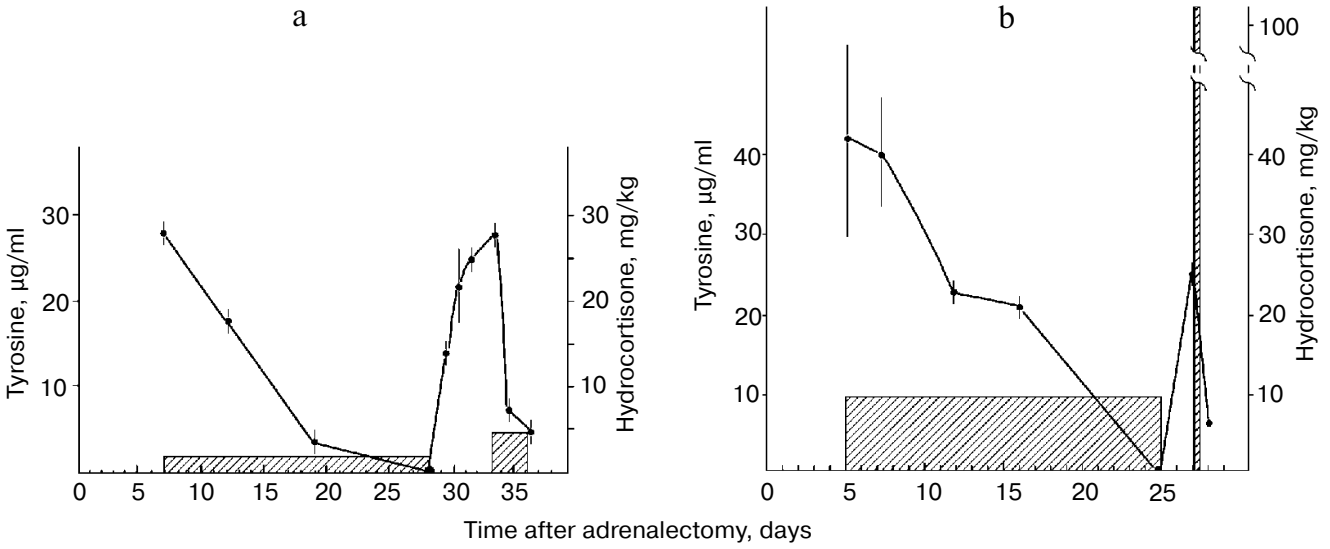


Fig. 2. Changes in tyrosine content in blood of adrenalectomized rats subjected to daily injections of hydrocortisone (hatched), upon their withdrawal and recommencement. a) Five animals, hydrocortisone injections in the dose of 2 mg/kg body weight; b) 10 animals, hydrocortisone injections in the dose of 10 mg/kg body weight.

sponded to the fall of corticosterone content reported in the literature [122] and to the greatest depth of its tissue deficiency [125]. Then the tyrosine content began to decrease and became normal on the 10th day after the operation, when the adrenalectomized rats "caught up" with the non-operated control rats also in body weight (Fig. 1).

In two series of the experiment, starting from the 5th and 7th days after the adrenalectomy, rats were injected daily with hydrocortisone in the doses of 10 and 2 mg/kg body weight (these doses approximately correspond to the maximal dose 80 and supporting dose 15 mg of prednisolone (PS) per day used in patients with non-endocrine diseases). Hydrocortisone injections during 20 days resulted in a decrease in tyrosine content to undeterminable values, and withdrawal and recommencement of the injections were accompanied, respectively, by the increase and decrease in the tyrosine content in the rat blood (Fig. 2).

Thus, under conditions of relative physiological rest blood tyrosine content in adrenalectomized rats inversely depended on the entrance of GCs – endogenous corticosterone or exogenous hydrocortisone.

The response of adrenal cortex and changes in blood tyrosine were followed in rats subjected to 30-min immobilization stress (Fig. 3) [121]. The content of 11-oxycorticosteroids in blood of the control rats was initially 20.4 μg/100 ml and increased in response to the immobilization to 68 and 32 μg/100 ml, respectively, 30 min and 4 h after the beginning of the exposure (the determination was performed in the serum pool from five animals). In the adrenalectomized rats on the 10th day after the surgery the blood content of 11-oxycorticosteroids before the immobilization was 8.7 μg/100 ml and could not be determined 30 min and 4 h after the beginning of the exposure.

The blood content of tyrosine before the immobilization was, on average, similar in the control and adrenalectomized rats (from 17.4 ± 4.8 to 23.6 ± 2.6 μg/ml in different series of the experiment). In the control rats the immobilization led to decrease in the tyrosine content: 4 h after the beginning of immobilization it was ~75% and after 22 h it was ~50% of the initial value. In the adrenalectomized rats the 30-min immobilization led to increase in the tyrosine content to ~115

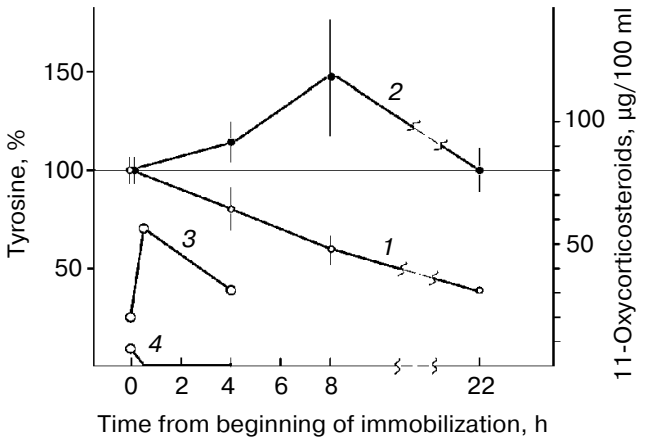


Fig. 3. Changes in content of tyrosine (*1*, *2*) (in percent of initial level) and 11-oxycorticosteroids (*3*, *4*) in blood of control (*1*, *3*) and adrenalectomized (*2*, *4*) rats after 30-min immobilization stress.

and ~150% of the initial value, respectively, 4 and 8 h after the beginning of the exposure.

Thus, increase in content of endogenous corticosteroids in response to immobilization stress was accompanied by decrease in blood tyrosine content, whereas in the absence of normal adrenocortical response the tyrosine content increased.

Adrenogenital syndrome (AGS) (synonyms: congenital adrenal hyperplasia, congenital virilizing adrenal cortex dysfunction) in children was chosen as a clinical model [126] because it seems to be the only situation allowing a physician to control the entrance of GCs into the organism and to some degree assess the adequacy of the hormonal provision to the organism's requirements. This disease is caused by the genetically determined deficiency of GC biosynthesis enzymes in the adrenal cortex, most frequently of 21β-hydroxylase, and a resulting shift to synthesis of androgens, mainly dehydroxyepiandrosterone. The decreased production of GCs induces an increased synthesis of ACTH in the adenohypophysis, and this leads to the permanent stimulation of the adrenal cortex and a surplus synthesis of androgens [127, 128]. The excess of androgens is displayed by a specific clinical picture: an abnormal structure of external sex organs, an accelerated body growth with an overdevelopment of masculine type muscles during the first years of life, and the early arresting of growth because of a premature ossification of tubular bones and closing down the growth zones ("the bone age is ahead of the passport one"), etc. In girls a pseudohermaphroditism picture is developed.

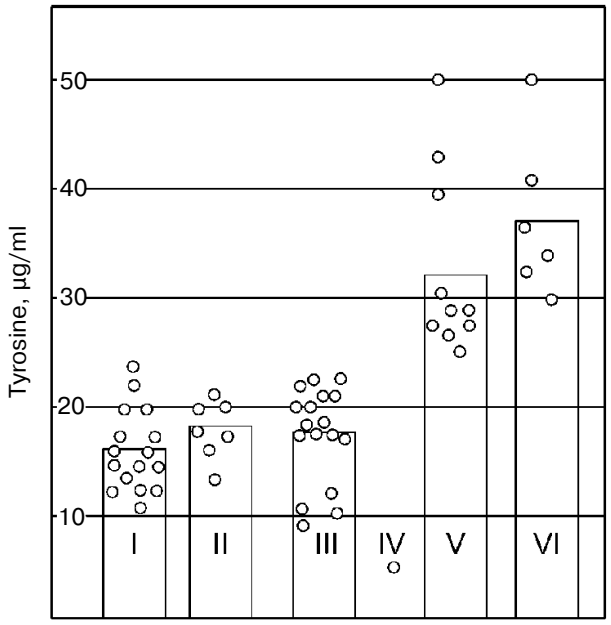


Fig. 4. Tyrosine content in blood of healthy donors and of children with adrenogenital syndrome. The columns present arithmetic means for the corresponding groups; the circles show individual values. I, healthy adults; II, healthy children; III, patients with complete clinical compensation; IV, a patient with overdosed GCs; V, patients with incomplete clinical compensation because of irregular treatment; VI, untreated patients.

Lifelong substitution GC therapy is the only pathogenetically reasonable treatment of this disease [129-132]. GC preparations break the vicious circle: recompensing the shortage of endogenous GCs they inhibit synthesis of ACTH responsible for the overproduction of androgens. The GC treatment must be started as early as possible after birth, and the appropriate GC dose can provide normal physical and sexual maturation of the affected children according to their genetic sex, with a possibility of normal pregnancy and labor in females. The choice of GC preparation dose must be strictly individualized, and matching an adequate dose is the most difficult and crucial stage. And depending on age and clinical manifestations, the daily dose of PS can vary from 2.5 to 15 mg. Overdosing GCs especially during the first three years of life can result in the growth arrest and usual complications of GC therapy. A temporary increase in the GC dose is recommended in the case of intercurrent diseases.

In children with AGS changes in the clinical picture – the rate and character of growth, ossification, and sexual maturation – allows a physician to make a relatively unbiased estimate of the correctness of the individual dose. In adult patients with AGS and in other conditions which require substitution GC therapy – after bilateral adrenalectomy or in chronic hypocorticism of various etiology – there are no clinical or laboratory determinants which would be helpful for monitoring appropriateness of the GC dose [133].

Our study [126] was performed in the Pediatric Department of the Institute of Experimental Endocrinology and Chemistry of Hormones, Russian Academy of Medical Sciences. Tyrosine content was determined in blood samples taken in the morning on empty stomach from 38 children with AGS (33 girls and five boys in the age of 3-18-year-old). The data were compared with the clinical picture, which characterized the degree of compensation of the genetic defect (Fig. 4).

In seven healthy children of 7-13-year age the tyrosine content in blood was 17.8 ± 1.0 μg/ml. On the complete clinical compensation of the genetic defect in 17 patients the blood tyrosine content was in normal limits, whereas in a patient with signs of Cushing's syndrome it was below the normal value. In the untreated patients and in the incompletely compensated patients (because of irregular treatment) the blood tyrosine content was significantly increased (Fig. 4). In three patients with pronounced melanodermia the blood tyrosine content was normal (not shown), i.e. a part of the excessive tyrosine was not oxidized by the major pathway but was converted into melanin (Scheme). Prescribing GC preparations resulted in "whitening" of such patients. This observation is in agreement with the described in the literature normalization of skin color in patients with hypocorticism

upon taking GCs [134]. Determination of blood tyrosine was helpful for choosing the optimal dose (10 mg PS per day) for two untreated girls with AGS of 4- and 7-year age whose "bone age" corresponded to 10- and 12-13-year age, respectively.

In two patients with the complete clinical compensation a transient increase in the tyrosine content up to 29.5 and to 45.0 μg/ml was recorded during an intercurrent respiratory disease. Obviously this reflected an increased requirement for the hormones and justified the empirical recommendation "to increase the dose of GC preparations".

Thus, in the clinical model the normal blood content of tyrosine corresponded to the full-value provision with GCs, whereas the increased tyrosine content indicated a shortage of these hormones.

BLOOD TYROSINE AND GLUCOCORTICOIDS IN SYSTEMIC LUPUS ERYTHEMATOSUS

Tyrosine content was systematically determined in blood of patients with SLE and the results were compared with clinical and laboratory data and especially with the regimen of using GC preparations. The work was performed in the Clinics of Therapy and Occupational Diseases of the First Moscow Medical Institute [135, 136].

SLE is the damage of connective tissue grasping virtually all organ systems: joints, skin, seroses, kidneys, lungs, cardiovascular system, gastrointestinal tract, blood system, and central nervous system. Multiple antibodies are found in the patients' blood, so SLE is also considered to be a typical autoimmune disease. SLE occurs in women significantly more frequently than in men and the disease usually starts in the age range of 20-30 years.

SLE is the most striking example of using GC preparations in non-endocrine diseases [137-140]. Previously to using GCs the life duration of 84% of patients with SLE was no more than 3 years, and GC preparations have radically changed the fate of patients with this most severe collagenosis: at present the life duration of 90% of patients is no less than 10 years and in some cases the capacity for work can be retained. GC preparations are used in combination with cytostatics, nonsteroidal anti-inflammatory drugs, aminoquinoline preparations, etc. However, in all schemes of SLE treatment GCs remain basic preparations, especially on generalization of the lupus process and on exacerbations.

Since 1966 life-threatening exacerbations of SLE are sometimes treated with so-called pulse-therapy – intravenous injection of very high doses of GC preparations, usually 1 g methylprednisolone per day during three days. This results in a rapid immunosuppressive effect but is often accompanied by various infections. Such regimen of pulse-therapy was decided historically, and, possibly lower doses of GCs would be similarly efficient [141, 142].

In SLE all side effects of long-term hormonal therapy occur: Cushing's syndrome, osteoporosis, aseptic osteonecrosis, generalization of latent infection, etc. In some patients so-called steroid dependence is developed that prevents even an insignificant lowering of the dose; the relapse of SLE ("withdrawal syndrome") makes impossible abolishing GC preparations in spite of serious complications.

And similarly to other diseases, in SLE there is no laboratory parameter allowing a physician to assess the patient's need in hormonal therapy, predict its efficiency, and control the dose.

We have observed 80 patients with SLE, 70 women and 10 men in the age range from 16- to 53-years-old, with 134 hospitalizations. The patients were not selected previously basing on case history and severity and character of the disease. Tyrosine content was determined in blood samples, which were taken for biochemical analysis (usually once during 7-14 days). Determination of tyrosine and *p*OPP in blood and urine revealed that the tyrosine content in blood increased because of disturbance of its transamination [143].

Tyrosine content in blood during the observation period was compared with clinical and laboratory data recorded in the case histories after the patients' discharge from the hospital, i.e. a kind of retrospective experiment was performed. Special attention was given to the regimen of GC therapy.

Changes in blood tyrosine content upon prescribing or increasing the dose of GC (40-60 mg/day calculated for PS) because of the disease exacerbation were followed in 32 patients with SLE. The comparison of the case histories and tyrosinemia revealed that 20 patients were given GCs on the background of significantly increased tyrosine content (49.1 ± 0.8 as compared to 16.2 ± 0.9 μg/ml in 16 healthy donors). The clinical and laboratory parameters were significantly improved in 17 of these patients, and in 13 of them this improvement was accompanied by a decrease in the blood tyrosine content outstripping appearance of signs of Cushing's syndrome; in four patients with pronounced liver damage hypertyrosinemia remained. It should be noted that the "improvement" in all cases concerned the disease itself and the side effects were considered as inevitable.

In 12 patients with SLE GC preparations were prescribed on the background of normal tyrosine content and were ineffective in nine of them, with a very rapid appearance of side effects in four patients. Some improvement was recorded in three patients, possibly, due to other drugs given concurrently: immunosuppressors, antibiotics, anti-allergic agents, heparin, etc.

Clinical steroid-dependence was characterized by a dramatic increase in the tyrosine content on attempts to even slightly lower the dose of GCs; short-term increas-

es ("splashes") in blood tyrosine were also observed under different stress conditions: intercurrent infection, difficult diagnostic procedure, reaction to a new drug, etc.

Figure 5 presents typical real situations of using GC preparations in patients with SLE compared with changes in the blood tyrosine content.

Let us try to imagine monitoring of hormonal therapy based on changes in blood tyrosine content. Patient M. (Fig. 5a) obtained 30-40 mg PS during two months. It would have been better to give her 40 mg (and not 30 mg!) earlier and to start the dose lowering on the normalization of blood tyrosine, i.e. the course of hormonal therapy would be shorter. Patient V. (Fig. 5b) had no need in GCs, but it was reasonable to give her a short-time hormonal "coverage" during the diagnostic procedure. In patient K. (Fig. 5c), the favorable effect of GCs was over within 10 days that was proved by the early development of complications at the normal tyrosine content. The "withdrawal syndrome" in patient P. (Fig. 5d), induced by the forced abolishment of GC, was associated with the dramatic rise in blood tyrosine. But considering the low blood content of tyrosine during the previous hospitalization, it would be reasonable to decrease the supporting dose the year before (may be even try to abolish GCs by the alternate-day scheme).

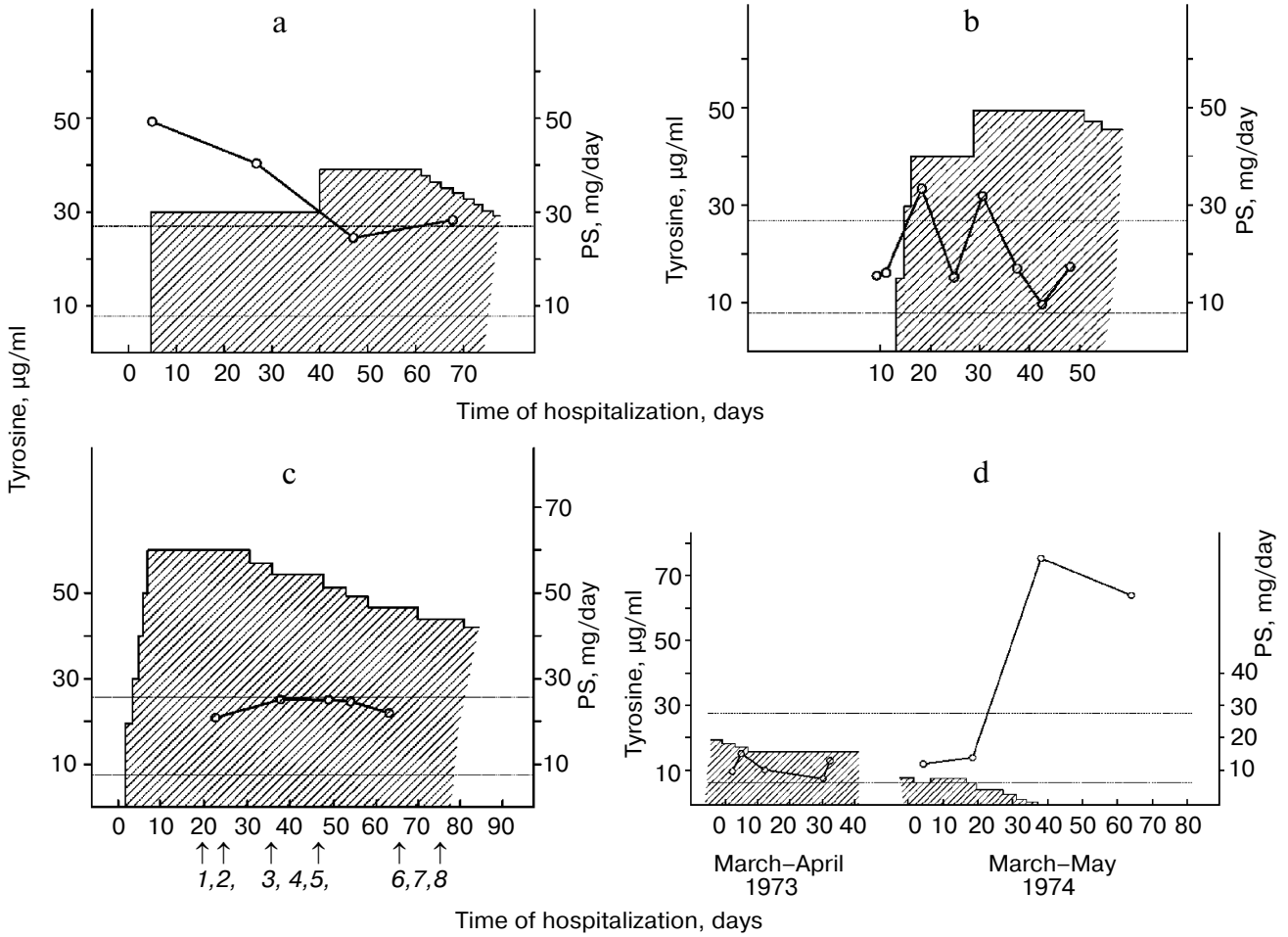


Fig. 5. Tyrosine content in blood of patients with SLE and doses of GC preparations (hatched). The dotted lines show normal limits of tyrosine content. a) A successful course of GC therapy. Patient M., 27 years old, aggravation of chronic SLE. She was given PS (30 mg/day) and antibiotics because of catarrhal state, after 20 days the immunologic activity was still present, and in 15 days the dose of PS was increased to 40 mg/day. This resulted in a pronounced improvement a week later, along with normalization of tyrosine content. Dose lowering was started after 20 days. b) A GC course without effect. Patient V., 26 years old, insolation-caused generalization of chronic SLE. She was prescribed with PS (40 mg/day), because of absence of effect 14 days later the dose of PS was increased to 50 mg/day and replaced by dexamethasone. After 1.5 months of inefficient hormonal therapy an improvement was reached by using azathioprine and heparin. Blood tyrosine during the hospitalization was in the limits of 10.0-17.5 μg/ml, tyrosine "splashes" were observed on a diagnostic procedure and on a hypertension crisis. c) Another collagenosis – patient K., 35 years old, with acute dermatomyositis. Upon excluding blastematosis, she was given PS (60 mg/day). Her conditions was improving within 10 days, and from the 14th day side effects (shown by arrows) began to appear, in particular: *1*) *acne vulgaris*; *2*) arterial hypertension; *5*) pronounced Cushing's syndrome; *8*) psychosis. d) "Withdrawal syndrome" in patient P., 22 years old, subacute SLE. Activation of SLE upon the forced abolishment of GCs because of development of aseptic osteonecrosis of femoral bone heads.

The course of hormonal therapy performed by Dr. I. A. Borisov under the control of blood tyrosine content did not lead to complications, although all previous courses in this patient were associated with a rapid development of side effects [136].

Thus, in patients with SLE GCs were favorable being prescribed at the increased content of blood tyrosine, i.e. under conditions of insufficient hormonal provision. Normalization of the blood tyrosine was ahead of appearance of hypercorticism manifestations, i.e. the therapeutic effect of GC preparations seemed to be exhausted on achievement of the adequate hormonal provision. Prescribing GCs at normal tyrosine content, i.e. under sufficient hormonal provision, inevitably resulted in a rapid development of side effects.

It is difficult to imagine modern clinical medicine without hormonal preparations and especially without GCs. GC preparations are used in all fields of medicine, and most frequently they are used not to compensate a shortage of the corresponding endogenous hormones but as the most effective anti-inflammatory drugs also possessing anti-allergic, antitoxic, and anti-shock effects.

However, GC preparations retain the specific hormonal features: similarly to their natural prototypes they control virtually all metabolic and physiological processes and just their hormonal features cause nearly inevitable side effects.

Despite the vital importance of natural GCs that are essentially responsible for supporting homeostasis under different conditions and play a decisive role during stress situations or in disease, routine clinical and laboratory methods cannot reveal what level of adrenocortical activity would be optimal for a given subject in a given situation. It is also unclear what dose of GC preparations is optimal for a given patient. For GCs there is no parameter similar to blood glucose content for insulin, which would characterize the effect of GCs on metabolism and tissue provision with these hormones.

Tyrosine content in blood, which under real conditions is mainly determined by the activity of the GC-dependent hepatic enzyme TAT seems promising as such a parameter. In many works hepatic TAT activity is used as an etalon in *in vitro* and *in vivo* studies on the GC action mechanisms. In particular, hepatic TAT activity is used along with parameters of the anti-inflammatory activity to characterize efficiency of new GC preparations [86] and also of steroidal and non-steroidal ligands of GC receptors [30, 87].

However, TAT cannot be used for clinical purpose because, first, it is a liver enzyme virtually undeterminable in blood and, second, TAT activity depends on the amount of GCs, and this prevents the assessment whether the hormonal provision corresponds the organism's needs.

Experimental data and clinical observations presented in this article show that tyrosine content in blood is promising as a representative index of the organism's provision with GCs. Note also that in a healthy human the blood tyrosine content is rather constant, i.e. is a specific personal parameter [103] and, thus, can be considered a parameter of homeostasis.

Introduction of such a parameter in clinical and laboratory practice would be useful for filling a very significant gap in characteristics of adrenocortical status of the organism because it can show whether the hormonal provision is adequate to the organism's needs. This parameter can be used for determination of requirements in GC preparations in both substitution therapy and non-endocrine diseases (on taking into account functional activity of the liver!). Determination of blood tyrosine content is helpful for individualizing the approach for prescribing these unique drugs, predicting their efficiency, and correcting the dose in due time. Thus, in patients with bronchial asthma tyrosine content was significantly increased during the period of attacks (along with an increased blood content of cortisol!) [144], and on this background GC preparations resulted in a rapid effect, whereas an abstention from them was associated with a slower reaching the remission [145].

The comparison of clinical effects of GCs in SLE with their regulatory influence on the blood tyrosine content suggests that the therapeutic action of GC preparations is not conditioned by an exclusive combination of pharmacological properties but by their ability to act through the natural pathways of GC hormones and normalize the course of the GC-dependent processes. It seems "that there is no fundamental distinction between the physiological and pharmacological effects of glucocorticoids and that their inhibitory actions on inflammatory and immune responses are concerned with modulating the body's defense mechanisms" [146]. In particular, selective ligand complexes with GC receptors act through the same pathways as natural GC hormones or "classical" GC preparations.

Apparently, using GC preparations is reasonable and effective as far as it can reproduce an optimal and full-value response of the adrenal cortex of a given person in disease or in a stress situation. The uniqueness and effectiveness of GC preparations seem to be due just to their use in the role of their natural prototypes according to their direct purpose; thus, their use in non-endocrine diseases can be considered as compensating substitution. This concept seems also to be true for using GC preparations in acute infections, shock, surgical operations, etc.

I am deeply grateful to Academician L. A. Piruzyan for his constant interest in the work, to I. M. Parkhomenko for discussion on the manuscript, and to K. L. Slavina and R. A. Byba for their help in designing the figures.

REFERENCES

1. Hench, P. B., Kendall, E. C., Slocumb, C. H., and Polley, H. F. (1949) *Proc. Staff Meetings Mayo Clin.*, **24**, 181-197.
2. Hench, P. B., Kendall, E. C., Slocumb, C. H., and Polley, H. F. (1950) *Arch. Int. Med.*, **85**, 545-666.
3. Salassa, R. M., Bennet, W., Keating, F. R., Jr., and Sprague, R. G. (1953) *JAMA*, **152**, 1509-1514.
4. Albeaux-Fernet, M., and Gelinet, M. (1960) *Sem. Hop. Paris*, **36**, 3401-3409.
5. David, D. S., Grieco, M. H., and Cushman, P., Jr. (1970) *J. Chron. Dis.*, **22**, 637-670.
6. Glyn, J. H. (1957) *Cortisone Therapy Mainly Applied to the Rheumatic Diseases*, N. Y.
7. Liddle, J. W. (1961) *Clin. Pharmacol. Ther.*, **2**, 615-635.
8. Boumpas, D. T., Chrousos, G. P., Wilder, R. L., Cupps, T. R., and Balow, J. E. (1993) *Ann. Int. Med.*, **119**, 1198-1208.
9. Strachunskii, L. S., and Kozlov, S. N. (1997) *Glucocorticoid Preparations. Methodical Handbook* [in Russian], Smolensk.
10. Chrousos, G. P. (2001) in *Endocrinology and Metabolism*, McGraw-Hill, N. Y., pp. 609-632.
11. Kirwan, J., and Power, L. (2007) *Arthr. Res. Campain*, October 2007, No. 13.
12. Richter, B., Neises, G., and Clar, C. (2002) *Endocrinol. Metab. Clin. North. Amer.*, **31**, 751-778.
13. Hopkins, R. L., and Leinung, M. C. (2005) *Endocrinol. Metab. Clin. North. Amer.*, **34**, 371-384.
14. Pisu, M., James, N., Sampsel, S., and Saag, K. G. (2005) *Rheumatology*, **44**, 781-788.
15. Schacke, H., Docke, W. D., and Asadullah, K. (2002) *Pharmacol. Ther.*, **96**, 23-43.
16. Davies, J. M., III, Maradit, K. H., Crowson, C. S., Nicola, P. J., Ballman, K. V., Therneau, T. M., Roger, V. L., and Gabriel, S. E. (2007) *Arthritis Rheum.*, **56**, 820-830.
17. Woolf, A. D. (2007) *Curr. Opin. Int. Med.*, **6**, 544-549.
18. McDonough, A. K., Curtis, J. R., and Saag, K. G. (2008) *Curr. Opin. Rheumatol.*, **20**, 131-137.
19. Kavanagh, R. J., and Kam, P. C. A. (2001) *Br. J. Anaesth.*, **86**, 110-119.
20. Paul-Clark, M. J., Roviezzo, F., Flower, R. J., Cirino, G., Del Soldato, P., Adcock, I. M., and Perretti, M. (2003) *J. Immunol.*, **171**, 3245-3252.
21. Linker, R. A., Weller, C., Lohder, F., Mohr, A., Schmidt, J., Knauth, M., Metselaar, J. M., and Gold, R. (2008) *Exp. Neurol.*, **211**, 397-406.
22. Barnes, P. J. (1998) *Clin. Sci.*, **94**, 557-572.
23. Heasman, S. J., Giles, K. M., Ward, C., Rossi, A. G., Haslett, C., and Dransfield, I. (2003) *J. Endocrinol.*, **178**, 29-36.
24. Rhen, T., and Cidlowski, J. A. (2005) *N. Engl. J. Med.*, **353**, 1711-1723.
25. Kulinsky, V. I. (2007) *Biochemistry (Moscow)*, **72**, 595-607.
26. Buttgereit, F., Straub, R. H., Wehling, M., and Burmester, G.-R. (2004) *Arthritis Rheum.*, **50**, 3408-3417.
27. Song, I.-H., Gold, R., Straub, R. H., Burmester, G.-R., and Buttgereit, F. (2005) *J. Rheumatol.*, **32**, 1199-1207.
28. Newton, R., and Holden, N. S. (2007) *Mol. Pharmacol.*, **72**, 799-809.
29. Lowenberg, M., Stahn, S., Hommes, D., and Buttgereit, F. (2008) *Steroids*, **73**, 1025-1029.
30. Schacke, H., Schotelius, A., Docke, W.-D., Strelke, P., Jaroch, S., Schmees, N., Rehwinkel, H., Hennekes, H., and Asadullah, K. (2004) *Proc. Natl. Acad. Sci. USA*, **101**, 227-232.
31. Schacke, H., Berger, M., Rehwinkel, H., and Asadullah, K. (2007) *Mol. Cell. Endocrinol.*, **275**, 109-117.
32. Coghlan, M. J., Jacobson, P. B., Lane, B., Nakane, M., Lin, C., Elmore, S. W., Kym, P. R., Luly, J. R., Carter, G. W., Turner, R., Tyree, C. M., Hu, J., Elgort, M., Rosen, J., and Miner, J. N. (2003) *Mol. Endocrinol.*, **17**, 860-869.
33. Miner, J. N., Ardecky, B., Benbatoul, K., Griffiths, K., Larson, C. J., Mais, D. E., Marschke, K., Rosen, J., Vajda, E., Zhi, L., and Negro-Vilar, A. (2007) *Proc. Natl. Acad. Sci. USA*, **104**, 19244-19249.
34. Buttgereit, F., Burmester, G.-R., and Lipworth, B. J. (2008) *Nat. Clin. Practice Rheumatol.*, **5**, 18-19.
35. Rass, I. T. (1978) *Patol. Fiziol. Eksp. Terapiya*, No. 2, 87-91.
36. Selye, H. (1936) *Nature*, **138**, 32.
37. Selye, H. (1937) *Endocrinology*, **21**, 169-188.
38. Root, B. (1955) *Cur. Res. Anaesth. Analg.*, **34**, 78-95.
39. Ingle, D. I., Nezamis, J. E., and Jefferies, J. W. (1949) *Amer. J. Physiol.*, **157**, 99-102.
40. Keul, J., Reindel, H., and Roskamm, H. (1963) *Int. Z. Angew. Physiol.*, **20**, 5-19.
41. Baxter, J. D., and Rousseau, G. G. (1979) in *Glucocorticoid Hormone Action*, Springer Verlag, Berlin, pp. 1-24.
42. Ingle, D. I. (1952) *J. Endocrinol.*, No. 4, 23-27.
43. Horwith, M. K., and Stokes, P. E. (1960) *Adv. Int. Med.*, **10**, 259-295.
44. Vegiopoulos, A., and Herzig, S. (2007) *Mol. Cell. Endocrinol.*, **275**, 43-61.
45. Jefferies, W. McK. (1994) *Med. Hypoth.*, **42**, 183-189.
46. O'Connor, T. M., O'Halloran, D. J., and Shanahan, F. (2000) *Q. J. Med.*, **93**, 323-333.
47. Jessop, D. S., and Harbuz, M. S. (2005) *Rheumatology*, **44**, 1097-1100.
48. Priftis, K. N., Paradimitriou, A., Nicolaidou, P., and Chrousos, G. P. (2008) *Trends Endocrinol. Metab.*, **19**, 32-38.
49. Goulding, N. J. (1998) *Br. J. Rheumatol.*, **37**, 477-483.
50. Wilckens, T. (1995) *Trends Pharmacol. Sci.*, **16**, 193-197.
51. Cancedda, C., Filaci, G., Puppo, F., Contini, M. P., and Indiveri, F. (2002) *Ann. N. Y. Acad. Sci.*, **966**, 49-63.
52. Fletcher, D. G., and Harkins, H. H. (1954) *Surgery*, **36**, 212-226.
53. Wright, A. R., and Krynski, B. (1962) *Arch. Surg.*, **85**, 180-183.
54. Yang, Y. X., and Lewis, J. D. (2003) *Semin. Gastrointest. Dis.*, **14**, 11-19.
55. Filaretova, L. P. (2007) *Neurosci. Behav. Physiol.*, **37**, 355-362.
56. Filaretova, L., Podvigina, T., Bagaeva, T., Bobryshev, P., and Takeuchi, K. (2007) *J. Pharmacol. Sci.*, **104**, 195-201.
57. Cope, C. L. (1966) *Brit. J. Med.*, **2**, 847-853.
58. Beisel, W. R., and Rapoport, M. I. (1969) *N. Engl. J. Med.*, **280**, 541-604.
59. Sampson, P. A., Brooke, B. N., and Winsone, N. E. (1961) *Lancet*, **7191**, 1377.
60. Salem, M., Tainsh, R. E., Bromberg, J., Loriaux, D. L., and Chernow, B. (1994) *Ann. Surg.*, **219**, 416-425.
61. Munck, A., Guyre, M., and Holbrook, N. J. (1984) *Endocrinol. Rev.*, **5**, 25-44.
62. Sapolsky, R. M., Romero, L. M., and Munck, A. U. (2000) *Endocrinol. Rev.*, **21**, 55-89.

63. Yeager, M. P., Guyre, P. M., and Munck, A. U. (2004) *Acta Anaestesiol. Scand.*, **48**, 799-813.
64. Kass, E. H., and Finland, M. (1958) *Adv. Int. Med.*, **1958**, 45-80.
65. Haleem, M. A., and Minton, S. A. (1966) *J. Trop. Med.*, **69**, 294-298.
66. Walzer, P. D., Powell, R. D., and Joneda, K. (1979) *Infect. Immunol.*, **24**, 939-947.
67. Knox, W. E. (1951) *Br. J. Exp. Pathol.*, **32**, 462-467.
68. Lin, E. C. C., and Knox, W. E. (1957) *Biochim. Biophys. Acta*, **26**, 85-88.
69. Kenney, F. T., and Flora, R. M. (1961) *J. Biol. Chem.*, **236**, 2699-2703.
70. Litwack, G., and Diamondstone, T. I. (1962) *J. Biol. Chem.*, **237**, 469-472.
71. Protasova, T. N. (1975) *Hormonal Regulation of Enzyme Activities* [in Russian], Meditsina, Moscow, pp. 26-43.
72. Boll, M., Weber, L. V. D., Font, M., and Stampfl, A. (1998) *Toxicology*, **126**, 127-136.
73. Rosen, F., and Nichol, C. A. (1963) *Vitam. Hormones*, **21**, 135-214.
74. Gelehrter, T. D. (1973) *Metabolism*, **22**, 85-100.
75. Baxter, J. D., and Tomkins, G. M. (1970) *Proc. Natl. Acad. Sci. USA*, **65**, 709-715.
76. Beato, M., Kalimi, M., and Feigelson, P. (1972) *Biochem. Biophys. Res. Commun.*, **47**, 1464-1472.
77. Munck, A. (1971) *Perspect. Biol. Med.*, **14**, 265-289.
78. Gelehrter, T. D., and McDonald, R. A. (1981) *Endocrinology*, **109**, 476-482.
79. Rivlin, R. S., and Melmon, K. L. (1965) *J. Clin. Invest.*, **44**, 1690-1698.
80. Thompson, E. B. (1979) in *Glucocorticoid Hormone Action*, Springer Verlag, Berlin, pp. 203-213.
81. Hashimoto, S., Schmid, W., and Schutz, G. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 6637-6641.
82. Sun, Y. N., DuBois, D. C., Almon, R. R., Pyszeznski, N., and Jusko, W. J. (1998) *J. Pharmacokinet. Biopharm.*, **26**, 619-648.
83. Grange, T., Cappabianca, L., Flavin, M., Sassi, H., and Thomassin, H. (2001) *Oncogene*, **20**, 3028-3038.
84. Sevaljevic, L., Isenovic, E., Vulovic, M., Macvanin, M., Zakula, Z., Kanazir, D., and Ribarac-Stepic, N. (2001) *Biol. Signals Recept.*, **10**, 299-309.
85. Hazra, A., Pyszeznski, N., DuBois, D. C., Almon, R. R., and Jusko, W. J. (2007) *J. Pharmacokinet. Pharmacodyn.*, **34**, 643-647.
86. Zimmermann, G. R., Avery, W., Finelli, A. L., Farwell, M., Fraser, Ch. C., and Borisy, A. A. (2009) *Arthritis Res. Ther.*, **11**, 12-26.
87. Ali, A., Thompson, C. F., Balkovec, J. M., Graham, D. W., Hammond, M. L., Quraishi, N., Tata, J., Einstein, M., Lan Ge, Harris, G., Kelly, T. M., Mazur, P., Pandit, S., Santoro, J., Sitlan, A., Shuanlin Wang, Williamson, J., Miller, D. K., Thompson, C. M., Zaller, D., Forrest, M. J., Carballo-Jane, E., and Luell, S. (2004) *J. Med. Chem.*, **47**, 2441-2452.
88. Braunstein, A. E. (1949) *Biochemistry of Amino Acid Metabolism* [in Russian], Medgiz, Moscow, pp. 270-293.
89. Knox, W. E. (1955) in *A Symposium on Amino Acid Metabolism*, John Hopkins Press, Baltimore, pp. 836-866.
90. Knox, W. E., and Goswami, M. N. D. (1961) *Ann. N. Y. Acad. Sci.*, **92**, 192-194.
91. Dagley, S., and Nicholson, D. E. (1973) in *Metabolic Pathways* [Russian translation], Mir, Moscow, pp. 224-228.
92. Scriver, C. R., Clow, C. L., and Lamm, P. (1971) *Am. J. Clin. Nutr.*, **24**, 826-890.
93. Wurtman, R. J., Rose, C. M., Chou, C., and Larin, F. F. (1968) *N. Engl. J. Med.*, **279**, 171-175.
94. Betheil, J. J., Feigelson, M., and Feigelson, P. (1965) *Biochim. Biophys. Acta*, **104**, 92-97.
95. Nemeth, S. (1978) *Horm. Metab. Res.*, **10**, 144-147.
96. Scriver, C. R. (1967) *Can. Med. Assoc. J.*, **97**, 1045-1101.
97. Cerone, R., Holme, E., Schiaffino, M. C., Caruso, U., Maritano, L., and Romano, C. (1997) *Acta Paediatr.*, **86**, 113-115.
98. Goldsmith, L. A. (1978) *Exp. Cell Biol.*, **46**, 96-113.
99. Natt, E., Kida, K., Odievre, M., Di Rocco, M., and Scherer, G. (1992) *Proc. Natl. Acad. Sci. USA*, **89**, 9297-9301.
100. Kainova, A. S. (1969) *Disorders of Tyrosine Metabolism in Rheumatism and in Experiment*: Author's abstract of Doctoral dissertation [in Russian], Moscow State University, Moscow.
101. Benkert, O., and Matussek, N. (1970) *Arzneimittel-Forsch.*, **7**, 905-906.
102. Armstrong, M. D., and Stave, U. (1973) *Metabolism*, **22**, 561-569.
103. Armstrong, M. D., and Stave, U. (1973) *Metabolism*, **22**, 821-825.
104. Levine, R. J., and Kohn, H. O. (1967) *J. Clin. Invest.*, **46**, 2012-2030.
105. Powell, L. W., and Axelsen, E. (1972) *Gut*, **13**, 690-696.
106. Nordlinger, B. M., Fulenwider, J. T., Ivey, J. L., Faraj, B. A., Ali, F. M., Kutner, M., Henderson, J. M., and Rudman, D. (1979) *J. Lab. Clin. Med.*, **94**, 832-840.
107. Belanger, R., Chandramohan, N., Misbin, R., and Rivlin, R. S. (1972) *Metabolism*, **21**, 855-865.
108. La Du, B. N., and Zannoni, V. G. (1961) *Ann. N. Y. Acad. Sci.*, **92**, 175-191.
109. Clayton, T. A., Lindon, J. C., Everett, J. R., Charuel, C., Hanton, G., Le Net, L. L., Provost, J. P., and Nicholson, J. K. (2007) *Arch. Toxicol.*, **81**, 201-210.
110. Nishimura, N., Yasui, M., Okamoto, H., Kanazawa, M., Kotaka, Y., and Shibata, Y. (1958) *Arch. Dermatol.*, **77**, 255-262.
111. Nishimura, N., Maeda, K., Yasui, M., Okamoto, H., Matsukawa, M., and Toshina, H. (1961) *Arch. Dermatol.*, **83**, 644-652.
112. Grupper, C. H., Gonnard, P., and Legrand, J. C. (1959) *Bull. Soc. Franc. Derm. Syph.*, **66**, 505-514.
113. Kainova, A. S., and Kwyatkovskaya, A. N. (1965) *Terap. Arkhiv*, No. 11, 44-52.
114. Goncharik, L. A., and Goncharik, I. I. (1972) *Dokl. Akad. Nauk BSSR*, **16**, 374-377.
115. D'yachkova, A. Ya., and Kislyak, N. S. (1962) *Vopr. Med. Khim.*, No. 2, 144-149.
116. Legenchenko, M. I., Merezhinskii, V. M., and Goncharik, L. A. (1974) *Pediatriya*, No. 6, 50-54.
117. Usvatova, I. Ya., Abramovich, A. B., Savchuk, B. D., and Lelish, G. T. (1974) *Sov. Med.*, No. 5, 31-35.
118. Kainova, A. S. (1974) *Vopr. Revmat.*, No. 1, 68-73.
119. Christiansen, J. J., Djurhuus, C. B., Gravholt, C. H., Iversen, P., Christiansen, J. S., Schmitz, O., Weeke, J.,

Jorgensen, J. O. L., and Moller, N. (2007) *J. Clin. Endocrinol. Metab.*, **92**, 3553-3559.
120. Rass, I. T. (1980) *Dokl. Akad. Nauk SSSR*, **250**, 1497-1499.
121. Rass, I. T. (1983) *Byull. Eksp. Biol. Med.*, No. 3, 29-31.
122. Panov, A. N., and Shalyapina, V. G. (1968) *Probl. Endokrinol.*, No. 2, 75-77.
123. Lascano-Gonzales, J.-M. (1934) *C. R. Soc. Biol.*, **116**, 451-454.
124. Ptak, W. (1962) *Folia Biol.*, **10**, 131-135.
125. Buckingam, J. C., and Hodges, J. R. (1974) *J. Endocrinol.*, **63**, 213-222.
126. Rass, I. T., Kuznetsova, E. S., and Zhukovskii, M. A. (1979) *Pediatriya*, No. 9, 26-29.
127. Bonjiovanni, A. M., and Eberlein, W. R. (1961) *Metabolism*, **10**, 917-935.
128. Brooks, R. V. (1979) in *The Human Adrenal Gland*, Academic Press, London, pp. 67-92.
129. Zhukovskii, M. A., Buraya, T. I., and Kuznetsova, E. S. (1977) *Inborn Dysfunctions of Adrenal Cortex in Children* [in Russian], Meditsina, Moscow.
130. Lo, J. C., Schwitzgebel, V. M., Tyrrell, V. M., Fitzgerald, P. A., Kaplan, S. L., Conte, F. A., and Grumbach, M. M. (1999) *J. Clin. Endocrinol. Metab.*, **84**, 930-936.
131. Stikkelbroeck, N. M. M. L., van't Hof-Grootenboer, B. A. E., Hermus, A. R. M. M., Otten, B. J., and van't Hof, M. A. (2003) *J. Clin. Endocrinol. Metab.*, **88**, 3525-3530.
132. Hughes, I. A. (2007) *Horm. Res.*, **68**, Suppl. 5, 84-89.
133. Lukert, B. P. (2006) *J. Clin. Endocrinol. Metab.*, **91**, 793-794.
134. Snell, R. S. (1967) *Adv. Biol. Skin*, **8**, 447-466.
135. Rass, I. T., and Sura, V. V. (1974) *Terap. Arkhiv*, No. 9, 135-139.
136. Rass, I. T., Borisov, I. A., Nikishova, T. A., and Sura, V. V. (1977) *Terap. Arkhiv*, No. 8, 110-115.
137. Dubois, E. L. (ed.) (1966) *Lupus Erythematosus*, McGraw Hill, N. Y.
138. Schroeder, J. O., and Euler, H. H. (1997) *Drugs*, **54**, 422-434.
139. Ioannou, Y., and Isenberg, D. A. (2002) *Postgrad. Med.*, **78**, 599-606.
140. Goldblatt, F., and Isenberg, D. A. (2005) *Clin. Exp. Immunol.*, **140**, 205-212.
141. Badsha, H., and Edwards, C. J. (2003) *Semin. Arthr. Rheum.*, **32**, 370-377.
142. Giovanni, F., and Diamond, B. (2005) *Autoimmunity Revs.*, **5**, 111-113.
143. Rass, I. T. (1976) *Vopr. Revmat.*, No. 4, 21-23.
144. Rass, I. T., Bunyatyan, A. F., Kornev, B. M., and Turusina, T. A. (1978) *Terap. Arkhiv*, No. 12, 107-109.
145. Zaslavskaya, R. M., Babalova, M. T., and Rass, I. T. (1990) *Klin. Med.*, No. 6, 96-100.
146. Munck, A., and Guyre, P. M. (1986) *News Physiol. Sci.*, **1**, 69-72.