trical and anginin (they act predominantly on the activation of the plasmin and prostaglandin systems), the quantitative difference in their effectiveness points to a predominant role of the kallikrein-kinin system in the pathogenesis of allergic myocarditis.

Some decrease in the intensity of the morphological manifestations of experimental myocarditis also was found when such anti-inflammatory agents as butadione and mephenamic acid were used [4].

The results of these investigations are evidence that activation of the myocardial kinin system occurring during the first five weeks of development of the inflammatory process leads to marked local hyperproduction of kinin, an important factor in the development of allergic myocarditis. The activation of the plasma kinin system discovered previously at these same times of development of myocarditis [2] evidently leads to summation of the harmful action of the plasma and myocardial kinins on the structure and function of the heart. The manifestations of allergic myocarditis can be considerably reduced by inhibition of the kinin system of the plasma and myocardium.

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EFFECT OF ALLERGIC PROCESSES OF IMMEDIATE AND DELAYED TYPES ON THE INTENSITY OF CORTISOL METABOLISM IN THE GUINEA PIG LIVER

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The intensity of cortisol metabolism was studied during perfusion of the liver in situ with solutions containing different concentrations of cortisol. Under these circumstances metabolism was shown to take place chiefly in the direction of cortisone formation. With an increase in the cortisol concentration in the perfusion fluid the intensity of its metabolism in the liver tissue also increased. During anaphylactic changes in the liver tissue the intensity of conversion of cortisol into cortisone was reduced, indicating a disturbance of the oxidation of cortisol. In experimental allergic encephalomyelitis the intensity of cortisol metabolism also was reduced.

KEY WORDS: *Allergy; glucocorticoids; metabolism of cortisol in the liver.*

In allergic diseases the half-elimination time of injected cortisol is increased, evidence of delay in its metabolism [7, 8]. Experiments on dogs have shown that during allergic changes in the liver tissue the intensity of cortisol metabolism is reduced [3], whereas no such disturbance has been found in dogs' kidneys [4]. Marked changes in cortisone metabolism have also been discovered in the liver of guinea pigs [5]. However, the general principles governing the change in corticosteroid metabolism in the liver during allergic processes are not yet known.

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The object of this investigation was to study the metabolism of cortisol in the liver of guinea pigs during allergic processes of immediate and delayed types.

EXPERIMENTAL METHOD

Male guinea pigs (13 animals) were sensitized with normal horse serum (NHS) by a single subcutaneous injection in a dose of 0.08 ml; experimental allergic encephalomyelitis was produced in 12 animals by a single injection of 0.15-0.2 ml of an encephalitogenic mixture consisting of rabbit spinal cord and complete adjuvant into the four footpads.

To study the intensity of cortisol metabolism in the liver of the guinea pigs the method of perfusion of the organ in situ through the portal vein with Tyrode solution containing 1.2% dextran, to which different concentrations of cortisol were added, was used. The technique of perfusion was described previously [5]. The outflowing perfusion fluid was collected in batches for periods of 9 min each, and the quantity of residual unmetabolized cortisol in these batches was determined by a fluorometric method.

The fluorometric method included purification of the perfusion fluid twice with carbon tetrachloride, extraction with methylene dichloride, and purification of the extract with alkali and water, after which the extract was shaken with a fluorescent mixture. Fluorometry was carried out 10 min later on a fluorometer with an incandescent lamp as the source of light, using primary and secondary interference filters of 470 and 540 nm and a type FEU-17 recording instrument with mirror galvanometer [2].

As a control of the fluorometric method and also to estimate cortisol metabolites, chromatographic isolation of the metabolites was carried out in various systems of solvents, followed by their identification from their luminescence in UV light, their R_f values, the chromogen spectrum in sulfuric acid, alkaline fluorescence, and their reactions with orthophosphoric acid, tetrazolium blue, and phenylhydrazine. Quantitative estimation was based on absorption in UV light and the reaction of Porter and Silber. The intensity of metabolism was judged, depending on the object of the experiment, either from the change in the cortisol concentration in the outflowing perfusion fluid or the quantity of hormone metabolized in μg/min/100 g weight of liver.

EXPERIMENTAL RESULTS

In the experiments of series I on 18 intact guinea pigs the characteristics of cortisol metabolism under the chosen experimental conditions were investigated. During investigation of the effect of the duration of perfusion on the intensity of cortisol metabolism two periods were distinguished: in the first period, lasting 19-27 min, the intensity of metabolism was increased, but then it fell rapidly, and in the second period, lasting 9-10 h, it remained stable. Accordingly, the results obtained in the second period were used in the calculations. Changes in the intensity of cortisol metabolism depending on its concentration in the inflowing perfusion fluid were then studied. It was found that the intensity of metabolism of cortisol increased regularly with an increase in its concentration in the inflowing perfusion fluid up to 100 μg% (Fig. 1). Consequently, under the experimental conditions chosen, a passive self-regulatory mechanism existed in the liver. A further increase in the concentration of cortisol was not accompanied by any increase in its metabolism, evidently suggesting complete saturation of the activity of the enzymes metabolizing cortisol.

To ascertain the character of the metabolites formed in the liver the method of paper chromatography was used. In these experiments perfusion was carried out with cortisol in a concentration of 100 μg%. Several systems were used for chromatography. In Busch's B_5 system, only two spots were isolated from the outflowing perfusion fluid and these were identified as cortisol and cortisone. During cortisol metabolism in the guinea pig liver under conditions of perfusion in situ, the principal metabolite formed was thus cortisone, in agreement with observations of other workers [1, 6]. Quantitatively speaking it accounted for 40-50% of all cortisol metabolites and about 7-10% of the quantity of cortisol entering the liver. In A and B_3 systems, the substance applied remained at the starting line and no new steroids were isolated compared with system B_5. In a system of formamide and chloroform, only two spots were isolated just as in system B_5, and these also were identified as cortisol after its chromatographic isolation in different systems virtually coincided with the results of the fluorometric determination.

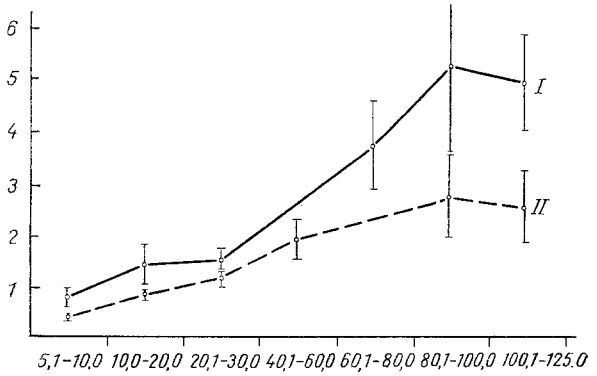

Fig. 1. Intensity of metabolism of cortisol in different concentrations in the inflowing perfusion fluid, in intact guinea pigs (I) and guinea pigs with encephalomyelitis (II). Ordinate, intensity of cortisol metabolism (in µg/min/100 g weight of liver, confidence limits shown for P=0.05); abscissa, ranges of cortisol concentration in inflowing perfusion fluid (in µg%).

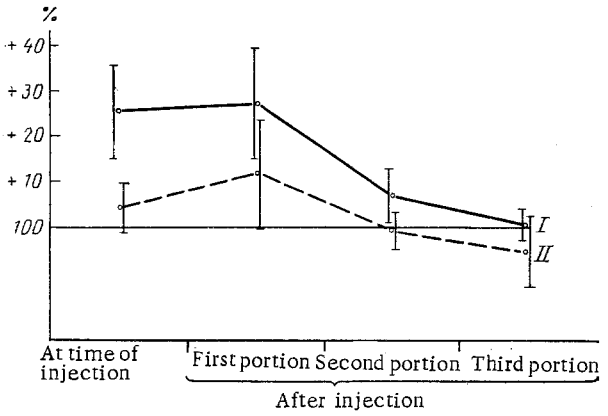

Fig. 2. Changes in intensity of cortisol metabolism in liver of intact (I) and sensitized (II) guinea pigs following injection of NHS antigen in a dose of 0.02-0.03 ml/ml perfusion fluid (confidence limits for P=0.05). Ordinate, changes in intensity of cortisol metabolism (in %); abscissa, Nos. of portions of outflowing fluid from time of injection of antigen.

In the experiments of series II the effect of NHS in concentrations of 0.01 to 0.05 ml/ml perfusion fluid on the rate of enzymic inactivation of cortisol was studied in the liver of 13 intact guinea pigs. The antigen was injected into the flow of perfusion fluid uniformly over a period of 9 min through a needle inserted into the rubber tube before the entrance into the liver. The cortisol concentration in NHS was determined beforehand and this value (the correction) was then subtracted from all the results. From 3 to 5 injections of NHS were tested on each liver. The results showed that injection of NHS into intact animals in concentrations of up to 0.02 ml/ml perfusion fluid had virtually no effect on the intensity of cortisol metabolism, but starting with a dose of 0.02 ml/ml and above, it stimulated cortisol metabolism significantly. The action of NHS reached a maximum after 9 min, i.e., in the first portion after injection of the antigen (Fig. 2). With an increase

in the NHS concentration the effect of its action also increased significantly ($P<0.05$-0.001). Special investigations showed that an increase in the duration of NHS administration was accompanied by an increase in the period of intensified cortisol metabolism in the liver tissue of intact guinea pigs.

The effect of anaphylactic changes in the liver tissue on the intensity of cortisol metabolism was next studied in 12 sensitized guinea pigs. The same concentrations of NHS as on intact guinea pigs were tested on these animals. A preliminary investigation showed that anaphylactic changes in the liver tissue were not accompanied by any liberation of fluorescent material. Injection of NHS into the flow of perfusion fluid was found to intensify cortisol metabolism in sensitized guinea pigs also, but the degree of intensification was lower than in the intact animals. However, a statistically significant difference between the intact and sensitized guinea pigs ($P<0.001$) was found only at the moment of injection of NHS in a concentration of 0.02-0.03 ml/ml perfusion fluid (Fig. 2). This is evidence that only the allergen–antibody complex formed by antibodies and antigen in a certain quantitative ratio had a harmful action.

In the experiments of series III changes in the intensity of cortisol metabolism were studied in 12 guinea pigs with encephalomyelitis. The perfused animals had marked pareses and paralyses. With all three concentrations of cortisol the intensity of its metabolism in the inflowing perfusion fluid was significantly lower ($P<0.001$) than in the intact animals (Fig. 1).

These investigations thus showed that in allergic processes of the immediate and delayed types the intensity of cortisol metabolism in the liver of guinea pigs is reduced compared with intact animals. However, the mechanism of this decrease differs depending on the type of the allergic process. As was shown previously [5], when anaphylactic changes are present in the liver tissue the conversion of cortisone into cortisol is reduced. Consequently, both the oxidation of cortisol at the C_{11} atom and the reduction of cortisone are inhibited simultaneously. This is evidence of a reduction in the activity of 11β-cortisol dehydrogenase and an expression of the harmful action of the allergen bound to cytophilic antibodies fixed on the liver cells.

In encephalomyelitis the pathological process is localized in the CNS, chiefly in the spinal cord; the conversion of cortisol into cortisone is reduced under these circumstances. Meanwhile as was shown previously [8], the conversion of cortisone into cortisol is considerably intensified under such conditions. Consequently, what takes place here is not a general depression of the activity of 11β-cortisol dehydrogenase, but a shift of this activity toward catalysis predominantly of reducing reactions. These changes must be regarded as protective and compensatory, aimed at increasing the cortisol concentration in the blood plasma and tissue fluid. They are evidently connected with changes in the ratio between oxidized and reduced forms of NADP, a cofactor for this enzyme.

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