5. Z. A. Ryabinina, Byull. Éksp. Biol. Med., No. 10, 94 (1973).
6. D. S. Sarkisov, A. A. Pal'tsyn, I. V. Popova, et al., Arkh. Patol., No. 1, 80 (1975).
7. N. I. Tsirel'nikov, L. I. Leont'eva, and G. S. Yakobson, in: Mechanisms of Regeneration and Cell Division (Proceedings of the Sixth Conference on the Study of Regeneration and Cell Division) [in Russian], Moscow (1971), pp. 195-196.
8. A. I. Éitingon, "Dependence of the biological action of some dihalogen derivatives of propane on their chemical structure," Candidate's Dissertation, Moscow (1969).
9. E. Bein, Zbl. Pharm. Pharmakother., 114, 583 (1975).
10. H. Gerhard, B. Schultze, and W. Maurer, Arch. Pathol. Anat. Abt. B, Zellpath., 14, 345 (1973).
11. L. A. Heppel, P. A. Neal, B. Highman, et al., J. Indust. Hyg., 28, 1 (1946).
12. T. A. Lawson and A. W. Pound, Brit. J. Exp. Pathol., 54, 583 (1974).
13. Y. Shimizu, C. Nagase, and K. Kawai, Ind. Health, 11, 48 (1973).

COMPARATIVE STUDY OF THE ACTION OF ETHIMIZOLE AND HYDROCORTISONE ON PROLIFERATIVE ACTIVITY AND PROTEIN SYNTHESIS IN EPITHELIAL CELLS OF THE TONGUE AND LIVER

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Unlike hydrocortisone, ethimizole stimulated mitotic activity of the epithelial cells of the tongue and liver 6 h after its administration. The decrease in the number of mitoses in the hepatocytes after 12 h was due to the action of both substances on DNA synthesis and not to a disturbance of the entry of the cells into mitosis. Stimulation of protein synthesis was detected by autoradiographic and biochemical methods following the action of hydrocortisone and ethimizole at the maximum of inhibition of mitosis.

KEY WORDS: *hydrocortisone; ethimizole; proliferative activity; protein synthesis.*

Glucocorticoids are known to inhibit tissue proliferation, including in the epithelium of the tongue [3] and of the intact and regenerating liver [2, 4, 8-11], considerably in rats. However, in most investigations no account was taken of the fact that the chemical structure of hydrocortisone and cortisone does not correspond to that of corticosterone, the principal corticosteroid produced by the rat adrenals [6], and that administration of exogenous hormones depresses the output of endogenous corticosteroids by the negative feedback principle [7]. It was therefore decided to make a comparative study of the action of hydrocortisone and ethimizole* (a substance which stimulates the pituitary—adrenal system 1 h after administration [5]) on proliferative activity of the epithelial cells of the tongue and liver.

Besides the marked antimitotic action of the corticosteroids, their other action, stimulation of protein synthesis [12], is well known. No reference could be found in the acces-

*Ethimizole is an original preparation obtained at the Institute of Experimental Medicine, Academy of Medical Sciences of the USSR (Leningrad) under the direction of Academician of the Academy of Medical Sciences of the USSR S. V. Anichkov and Corresponding Member of the Academy of Medical Sciences of the USSR N. N. Khromov-Borisov. (It is an alkylamide of inidazoledicarboxylic acid — Translator.)

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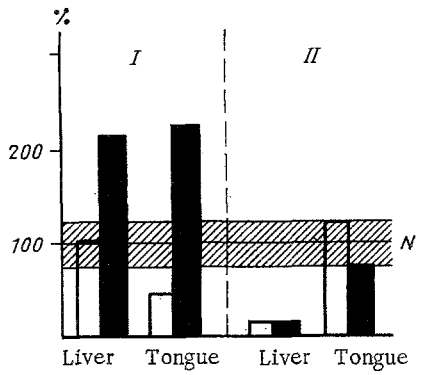

Fig. 1

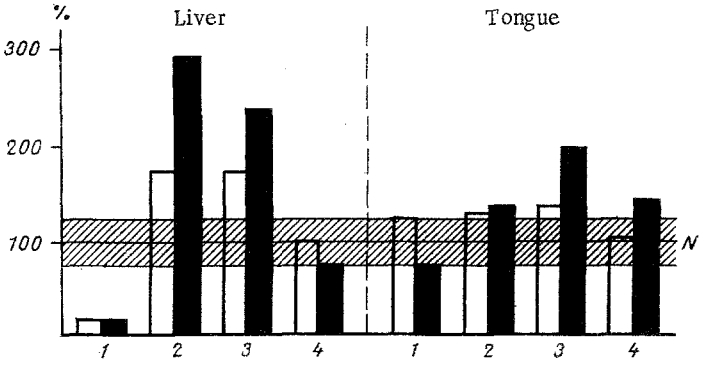

Fig. 2

Fig. 1. Mitotic index of epithelial cells of liver and tongue 6 (I) and 12 (II) h after injection of ethimizole (black columns) and hydrocorticone (white columns). Ordinate, mean mitotic index (in % relative to control). Shaded band indicates control with confidence limits.

Fig. 2. Protein metabolism and mitotic index in liver and tongue 12 h after injection of ethimizole (black columns) and hydrocortisone (white columns): 1) mitotic index; 2) mg protein/mg tissue; 3) counts/mg tissue; 4) counts/mg protein. Ordinate, protein metabolism and mitotic index (in % of control). Shaded band indicates control with confidence limits.

sible literature to a comparison of the action of these hormones on proliferation and protein synthesis, although such an approach is of considerable interest for it can provide data on the relationship between the two principal functions of cells, namely differentiation and proliferation.

This paper describes a comparative study of the action of ethimizole and hydrocortisone on proliferative activity and protein synthesis in the epithelial cells of the tongue and liver.

EXPERIMENTAL METHOD

Experiments were carried out on male noninbred albino rats weighing 200-350 g from the "Rappolovo" nursery. Between 11 a.m. and noon two-thirds of the liver of all the rats was removed by the method of Higgins and Anderson and the animals were divided into three groups. Forty-four hours after partial hepatectomy the animals of group 1 received hydrocortisone (5 mg/100 body weight), and the rats of group 2 received ethimizole (2 mg/100 g body weight). The animals of group 3 acted as the control. All the animals were killed 6 and 12 h after injection of labeled precursors: thymidine-^{3}H (specific activity 4.1 Ci/mmole) in a dose of 0.5 μCi/g body weight and methionine-^{35}S in a dose of 1 μCi/g. The number of mitotically dividing cells was counted in histological sections and the number of cells labeled with thymidine-^{3}H and methionine-^{35}S in histoautoradiographs. On the basis of these findings the mitotic and radiation indices and the number of tracks per conventional unit of area (50 μ^2) were calculated. Protein synthesis also was studied by a biochemical method.* The protein content was determined from the absorption at 280 nm in the SF-4a spectrophotometer and calculated from a standard curve. The radioactivity of the isolated proteins was determined with a Mark 2 scintillation counter. The specific radioactivity of the proteins was expressed in counts/min/mg protein and the specific radioactivity of the tissues in counts/min/mg tissue. The results were subjected to statistical analysis with the aid of the U and t criteria.

EXPERIMENTAL RESULTS

After injection of hydrocortisone (Fig. 1) differences in the response of the tissues were clearly revealed: In the epithelium of the tongue the mitotic activity fell after 6 h ($P < 0.01$) and in the liver after 12 h ($P < 0.005$). Unlike hydrocortisone, ethimizole stimulated cell division as early as after 6 h in both the liver and the tongue. This effect may

*The authors are grateful to V. S. Turovskii for advice and help with the biochemical investigations.

have been due to stimulation of cells which had completed DNA synthesis but had not yet started mitosis, i.e., cells in the R_2 phase of the mitotic cycle. After 12 h, ethimizole caused sharp depression of mitosis in the liver at a time when the level of mitosis in the eipthelium of the tongue was being restored. The action of ethimizole thus differed with respect to time.

Much light on the cellular mechanisms of inhibition of mitotic division of the hepatocytes was shed by the experiments with thymidine-^{3}H. Six hours after injection of the preparations, when hydrocortisone had not caused inhibition of mitosis and ethimizole had actually stimulated mitosis, entry of the cells into the phase of DNA synthesis was inhibited. In the control animals, for instance, the number of labeled hepatocytes was 55.5%, falling under the influence of ethimizole and hydrocortisone to 32.8% ($P = 0.025$) and 33.9% ($P < 0.05$), respectively. This effect was evidently due to blocking of the G_1-S periods. The decrease in the number of mitoses occurring after 12 h in the hepatocytes was therefore due to the action of the preparations on DNA synthesis and not to disturbance of entry of the cells into mitosis.

Protein synthesis was investigated 12 h after injection of the preparations, when both hydrocortisone and ethimizole strongly inhibited mitotic activity of the liver cells. The autoradiographic data revealed a statistically significant increase in the number of ^{35}S tracks above the hepatocytes: from 2.4 normally to 3.5 ($P < 0.025$) and 3.6 ($P = 0.01$) after treatment with hydrocortisone and ethimizole, respectively. Biochemical analysis of protein synthesis filled in the details of this autoradiographic picture. As Fig. 2 shows, ethimizole led to a greater and hydrocortisone to a smaller increase in the protein content per milligram of tissue. There was a corresponding increase in the number of counts per minute per milligram of tissue. The specific radioactivity of protein, however, remained at the control level. The increase in the number of tracks on the liver autographs could thus be explained, on the basis of the biochemical results, by protein synthesis, in agreement with data in the literature [11]. The results of the present experiments on the liver also show that protein synthesis is stimulated at a time of inhibition of proliferation. These findings are largely confirmed by the results of a comparison of protein synthesis and proliferation processes in the epithelium of the tongue. For instance, hydrocortisone did not depress the number of mitoses after 12 h and, naturally, protein synthesis remained at the control level. On the other hand, ethimizole caused very slight stimulation of protein synthesis in the tongue at a time of some depression of mitosis.

The cellular mechanisms of the stimulating action of ethimizole and hydrocortisone on protein synthesis can be explained, in the writers' view, in two ways: 1) by an increase in the number of cells performing the specific function, on account of a decrease in the number of proliferating cells; 2) by stimulatin of protein synthesis in differentiated cells which were outside the mitotic cycle at the time of action of the drugs.

LITERATURE CITED

1. O. I. Epifanova and V. V. Terskikh, Zh. Obshch. Biol., No. 4, 392 (1968).
2. T. A. Zaletaeva, Dokl. Akad. Nauk SSSR, 165, 421 (1965).
3. S. A. Ketlinskii, Arkh. Anat., No. 11, 76 (1974).
4. L. V. Orlova, Probl. Éndokrinol., No. 3, 69 (1976).
5. V. E. Ryzhenkov, T. A. Oletskaya, and N. S. Sapronov, Byull. Éksp. Biol. Med., No. 5, 50 (1973).
6. N. A. Yudaev, The Biochemistry of Steroid Hormones of the Adrenal Cortex [in Russian], Moscow (1956).
7. N. A. Yudaev, M. B. Lebedeva, and N. P. Zavyal'skaya, Probl. Éndokrinol., No. 6, 13 (1957).
8. J. C. Davis and T. A. Hyde, Cancer Res., 26, 217 (1966).
9. L. Desser-Wiest, Cell Tissue Kinet., 8, 1 (1975).
10. J. W. Guzek, Acta Endocrinol. (Copenhagen), 59, 10 (1968).
11. T. A. Hyde and J. C. David, Eur. J. Cancer, 2, 227 (1966).
12. F. J. Kenney, J. Biol. Chem., 237, 3495 (1962).