COMBINED ACTION OF THIOTEPA, OMAINE AND OF SOME HORMONAL PREPARATIONS ON THE EPITHELIUM OF MOUSE UTERINE CERVIX AND VAGINA

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In mice the epithelium of the uterine cervix and vagina is characterized by high proliferative activity and a characteristic double function, i.e. the ability at various phases of the cycle of differentiation to produce at one time stratified squamous and cornified epithelium and at other times a double prismatic layer forming mucous [1,2]. The same phenomena are found in these animals in the uterine cervix and vagina [1,4]. Therefore in making a choice of substances to act on the mouse uterine cervix and vagina it is best first to study the action of these substances on the normal epithelium of these regions.

The aim of the present investigation has been to study the action of thioTEPA and also the combined action of thioTEPA, omaine (colchamine) and certain hormonal preparations on the normal mouse uterine cervix and vagina.

METHOD

The experiments were carried out on white female mice of an impure strain aged about two months; some of the mice were first castrated. The preparations were introduced in lanoline on tampons of white cotton wool inserted into the vagina or introduced intraperitoneally as a suspension in physiological saline given six times per week (Table 1).

Each experiment lasted for 9-13 days. After the completion of the injections the mice were killed with chloroform. The bodies were fixed in 10% formalin. Uterus and vagina were embedded in a single block of paraffin. Whole coronal sections were cut so that in most preparations the different parts of the epithelium of the vagina and uterine cervix were shown. The sections were stained in hematoxylin-eosin, or in Heidenhain's hematoxylin-mucicarmine.

RESULTS

The injection of thioTEPA elicited the development of characteristic dystrophic changes in the basal layers of the epithelium of the uterine cervix and the vagina; the cell size increased and the nuclei stained less intensely with hematoxylin (large and very pale nuclei). The chromatin in the nucleus was distributed in the form of a few large clumps. In different animals and at different times the epithelium of the vagina and uterine cervix showed this effect to various extents. Sometimes the change was encountered comparatively infrequently, the epithelial layer appeared almost unchanged (slight degree of damage). In other cases in the basal layers numerous dystrophic cells were present. The intermediate portions of the layer seemed to have disappeared. Over the dystrophic altered cells of the basal layer there immediately appeared cornified elements belonging to the most superficial layers. Quite frequently in such cases the cornification took the form of a parakeratosis (moderate degree of damage). Finally, in many mice the whole of the epithelial layer was made up of dystrophically altered cells at various stages of degeneration and disintegration. Quite frequently there were an abnormally large number of leucocytic elements distributed diffusely or in clumps. These necrotic portions of the layer were separated from the underlying connective tissue, and desquamated, leading to the formation of small abscesses (severe damage).

TABLE 1. Doses and Details of Injection of the Preparations

Preparation	Method of administration	Value of single dose (in mg)	No. of injections in the different series	Total dose (in mg)
ThioTEPA	Into vagina	0.01-0.03	8-12	0.08-0.36
Colchamine	" "	0.02	6-12	0.12-0.24
Hexestrol	" "	0.15	6-12	0.9-1.8
Testosterone propionate	" "	1.5	6-11	9.0-16.5
	" "	0.25	6-11	1.5-2.75
Cortisone	Intraperitoneally	0.1-0.375	4-8	0.4-1.5

TABLE 2. Distribution of Mice According to the Extent of the Epithelial Damage

Experimental conditions	No. of mice	Dystrophic epithelial changes		
		Severe	Moderate	Mild
ThioTEPA	15	3	11	1
ThioTEPA and colchamine	9	5	4	—
ThioTEPA, colchamine, and oestrogen	31	—	10	21
Colchamine, oestrogen, and androgen	14	7	5	2
Colchamine, oestrogen, androgen, and cortisone (in vagina)	16	11	2	3
Colchamine, oestrogen, androgen, cortisone (in vagina and intraperitoneally)	14	11	2	1
Age control	14	—	—	—

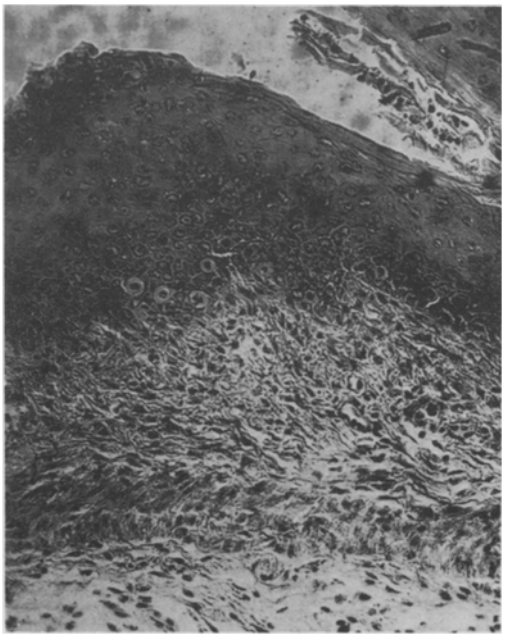
Simultaneous injection of thioTEPA and colchamine led to similar changes, except that in the preparations there could be seen also cells which were enlarged and whose nuclear substance had undergone disruption at the stage of metaphase (see Figure). The number of cells varied according to the preparations.

In experiments with simultaneous use of thioTEPA, colchamine and hexestrol the dystrophic changes were much less strongly shown; the epithelial layer was correctly formed, was of normal thickness, and well cornified. Only in the basal layers could occasional altered cells be found. With simultaneous injection of oestrogen and androgen, the action of the former preponderated. Almost everywhere on the preparations the epithelium consisted of stratified flat cornified cells, only in three cases were regions of metaoestral epithelium present. Also, dystrophic changes of various degrees of severity were present in the epithelial cells; they resembled the changes recorded after the injection of thioTEPA and colchamine.

The addition of cortisone aggravated the degree of damage. This affect was found both when the preparation was introduced on tampons, or when injected intraperitoneally. Finally the changes were particularly clearly shown when cortisone was introduced intraperitoneally or on a tampon. In certain cases the changes were particularly marked in the outgrowths of epithelium growing into the underlying connective tissue.

In control preparations (of mice of the same age) the epithelium showed no difference from normal, and was found to be at various stages of the oestrus cycle.

As can be seen from Table 2 the number of cases investigated histologically was 113. Use of thioTEPA alone or in conjunction with colchamine led to severe or moderate changes in 23 out of the 24 cases; when oestrogen is added there were no cases of severe damage, and moderate damage was found only in 10 of the 31 mice. When colchamine, oestrogen, and androgen were given simultaneously severe or moderate changes in the epithelium



Effect of nine simultaneous applications of thioTEPA and colchamine to mouse vaginal epithelium on the tenth day. In the basal layer of the epithelium there are "chains" of large dystrophically altered cells having a very pale cytoplasm and pyknotic nuclei. The epithelial damage is of moderate severity. Stain hematoxylin-eosin. Micrograph. Magnification 200.

occurred in 12 out of 14 mice, and when cortisone was added in 26 out of 30; in the latter case the number of rats with severe changes was the greatest (22 out of 30).

It is known that the introduction of colchamine into the vagina not only leads to "freezing" of the mitoses in metaphase but also exerts a toxic influence on cells at interkinase; this effect is shown by the development of characteristic dystrophic changes in the cells of the basal layer of the epithelium of the uterine cervix and vagina [1]. We observed similar changes when thioTEPA was used.

The small number of animals used for the experiment of does not enable us to relate the damaging influence of thioTEPA and the combination of thioTEPA with colchamine. The addition of androgen did not appreciably affect the influence. Hexestrol however blocked the toxic action of thioTEPA and colchamine. Thus, in animals who received a combination of thioTEPA colchamine, and oestrogen showed a much weaker damaging effect. Possibly in this case a certain degree of stimulation by oestrogen of the special epithelial activity is of significance [1,2].

With simultaneous injection of balanced doses of oestrogen and androgen again dystrophic changes were observed in the basal layers accompanied, in particular, by disturbance of the process of cornification (paraketosis) [3]. In our preparations oestrogen exerted the preponderant action (oestral reaction). Therefore the dystrophic changes observed in animals which received a combination of colchamine, oestrogen, and androgen, or a combination of these three preparations with cortisone must be attributed to the action of colchamine or to colchamine and cortisone. We found no reduction of the effectiveness of the preparation to be caused by oestrogen. Possibly the reason is to be found in the presence of androgen which is the antagonist of oestrogen. Possibly the same line of thinking may be applied to the enhancement of the damaging action of colchamine in experiments in which cortisone was given at the same time. This substance is known to exert an androgen-like action on the epithelium of the vagina and uterine cervix.

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