THE EFFECT OF HYPOPHYSECTOMY ON THE REACTIONS OF ANIMALS TO DIPHTHERIA TOXIN

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In research during recent years great attention has been paid to the question of the association between the hypohysis and the adrenal system. According to the theoretical views of some writers [3, 5, 7], the hypophyseal-adrenal system plays an important role in the development and outcome of many pathological processes, especially of an infectious nature. In a previous communication [1] we presented facts showing that the injection of lethal doses of diphtheria toxin 3-4 days after hypophysectomy does not cause the characteristic structural changes of diphtheria toxemia to develop in the internal organs. The question accordingly arose, whether this effect of hypophysectomy is maintained if the interval between the operation and the injection of diphtheria toxin is lengthened. Reports in the literature show that the reaction of animals to stimuli differs in character depending on the time elapsing after hypophysectomy [6].

The object of the present investigation was to study the effect of hypophysectomy on the development of structural changes in the internal organs at long intervals after the operation, and also to analyze the development of specific immunity in these conditions.

EXPERIMENTAL

To reproduce the disease, we used diphtheria toxin of batch No. 131, obtained from the N. F. Gamaleya Institute of Epidemiology and Microbiology, and for immunization we used an adsorbed diphtheria toxoid, prepared at the same institute, and containing 64 antitoxin units in 1 ml. Experiments were conducted on guinea pigs of both sexes, weighing 250-300 g, and the hypophysis was removed by the parapharyngeal route.

For the histological study of the structural changes in the organs and tissues, these were fixed in 12% neutral formalin. Sections were cut on a freezing microtome, and were then stained with hematoxylin-eosin and Sudan. The immunological indices (the antitoxin content of the blood serum) were studied by the usual biological method of Jensen, as modified by Khalyapina.

In the first series of experiments there were 19 guinea pigs (9 experimental and 10 control animals). Diphtheria toxin was injected into the right gastrocnemius muscle of the animals (0.003 ml of liquid toxin, diluted with 0.1 ml physiological saline) 11-12 days after hypophysectomy.

RESULTS

The animals of the experimental group began to die on the first, second and third days of the disease; the control guinea pigs died mainly on the fourth day. At postmortem examination the naked-eye changes in the organs were the same in the animals of the experimental and control groups, and consisted of slight hyperemia of the adrenals and the gastrocnemius muscle; the chambers of the heart were dilated and filled with blood clots, and the heart muscle was flabby. On microscopic study of the internal organs of the experimental and control guinea pigs the most marked changes were observed in the adrenal glands and the right gastrocnemius muscle.

We shall first consider the morphological picture in the animals of the control group, for the changes here were most conspicuous. In the cortical layer of the adrenal foci of necrosis and necrobiosis of various sizes were observed. In these areas the clear boundaries between the cells were absent.

The cytoplasm in some cases appeared as a homogeneous mass, and in others it was collected into granules. Some cells were without nuclei, while in others the nuclei were in a state of pyknosis or rhexis. In certain sections, in or near the areas of necrosis, we found small hemorrhages and foci of infiltration, consisting of fragments of cell nuclei, leukocytes, lymphocytes, and histiocytes. In the cortical layer of the adrenal, the content of lipids was reduced; lipids were distributed irregularly. Lipoid inclusions were most often preserved in the cells of the peripheral part of the zona fasciculata, forming a distinctive type of border of varying width, surrounding the adrenal. In most cases the lipoid materials were well preserved in the areas undergoing necrosis, whereas they were absent from the structurally unchanged cells; only in solitary cases were lipoid materials not found in the areas of necrosis. Between the muscle fibers of the heart small collections of leukocytes and lymphocytes were seen; in some sections nearly the whole muscle was infiltrated with leukocytes. The vessels were dilated and filled with erythrocytes; around them were seen perivascular infiltration and hemorrhages. In five cases, staining for lipids showed a diffuse lipoid degeneration of a group of muscle fibers. In the gastrocnemius muscle (the region where the toxin was injected) of three animals, small foci of necrosis, hemorrhage, and leukocytic infiltration were found. In all cases the muscle had undergone lipoid degeneration. No marked changes were observed in the liver and kidneys of the animals dying at this period.

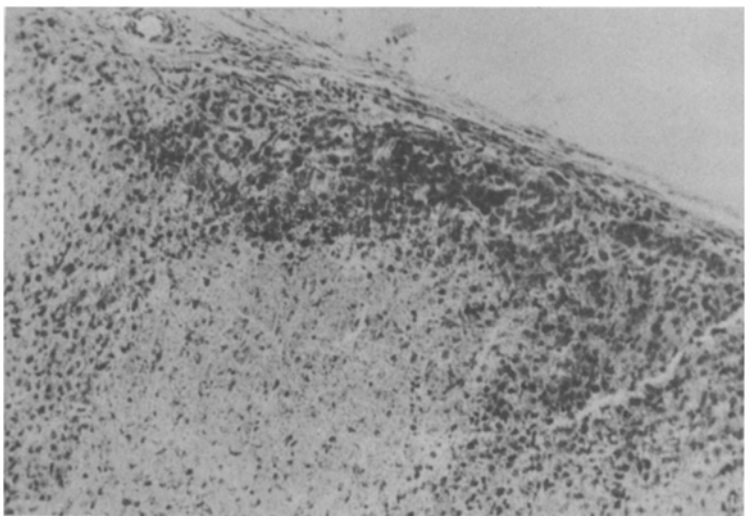

Fig. 1. Adrenal gland of a hypophysectomized guinea pig, dying on the third day after injection of diphtheria toxin. In the cortical layer of the gland are seen nonnucleated areas of necrosis. Photomicrograph.

The changes developing after injection of diphtheria toxin into hypophysectomized guinea pigs bore the same character as those in the control animals, but they were found less frequently and were less intensive. Microscopic examination of the adrenal glands of three hypophysectomized guinea pigs revealed foci of necrosis and necrobiosis in the cortical layer of the gland. These changes were localized more often in the right adrenal. The focal lesions were small and the vascular disturbances were ill defined (Fig. 1). In all cases a considerable decrease in the content of lipoid materials was observed in the adrenal cortex of the hypophysectomized animals, the lipids being preserved only in the cells of the peripheral part of the zona fasciculata in the form of a narrow border. In the medullary layer of the adrenal some cells were undergoing karyo-pyknosis. Hemorrhages and small areas of perivascular infiltration were present in the heart. Lipoid degeneration of the muscle was absent (Fig. 2). At the site of injection of the toxin into the gastrocnemius muscle, in a few cases small areas of infiltration were seen, consisting of collections of leukocytes, lymphocytes, and fibroblasts, and also of disintegrating cells; hemorrhages were also observed in the same areas. Only once were well marked necrosis and lipoid dystrophy of a group of muscle fibers seen, and in all the other cases lipoid inclusions were absent from the gastrocnemius muscle of the hypophysectomized guinea pigs in contrast to the control animals.

Thus after injection of diphtheria toxin into guinea pigs on the 11th-12th day after hypophysectomy, the changes developing in the organs of the experimental and control animals were similar in principle in their character, but in

the control animals they were encountered more often and were more intensive. It is interesting that in response to the injection of diphtheria toxin the content of lipoid matter in the adrenal cortex decreased in the animals of both groups; this manifestation of a nonspecific stress reaction demonstrates the stimulation of the hormonal function of the cortical layer of the adrenal gland. We must also point out the difference in the content of lipoid matter in the foci of necrosis observed in our experiments: in some cases the lipids were well preserved in the necrotic tissues, but in others, on the other hand, they had disappeared from the affected areas. This phenomenon may be explained by a disturbance of the circulation of the blood: if the necroses developed rapidly on account of a disturbance of the circulation, then the lipids could not be cleared completely by the blood flow; in those cases, however, when necrosis developed slowly, the lipoid inclusions could be removed. Subsequently (in the recovery period) the lipids were gradually restored only in the adrenal tissue preserved intact, whereas the damaged cells did not recover their lipoid matter.

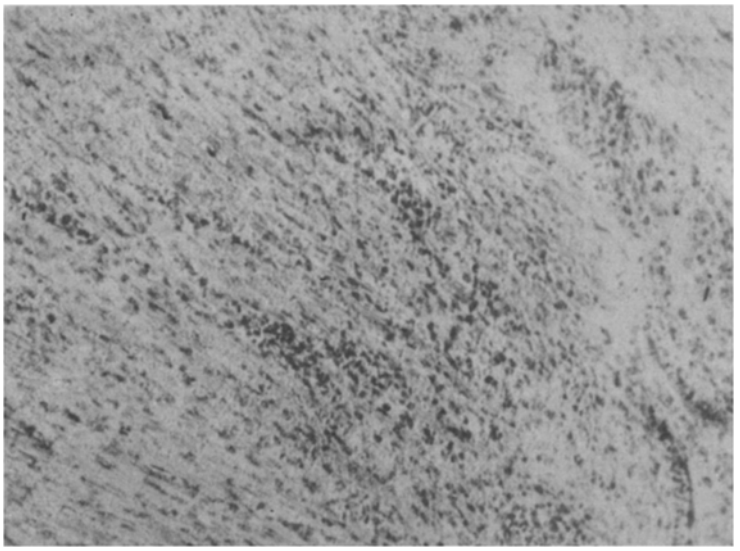

Fig. 2. Heart muscle of a hypophysectomized guinea pig, dying on the second day of the disease. Homorrhages and infiltration of leukocytes into the myocardium are seen. Photomicrograph.

TABLE 1. Antitoxin Content (in Antitoxin Units) in the Blood of Guinea Pigs at Various Times of Immunization with Diphtheria Toxoid in Hypophysectomized and Intact Animals (First Series of Experiments; M ± m)

Group of animals	After first injection			After second injection	
	10th day	21st day	42nd day	10th day	21st day
Experimental	0,002 (0,002±0)	0,120 (0,119±0,034)	0,225 (0,225±0)	2,750 (2,750±1,230)	3,700 (3,670±1,300)
Control	0,002 (0,001±0,173)	0,072 (0,165±0,092)	0,200 (0,200±0,030)	3,000 (3,000±1,040)	4,700 (4,688±0,277)

It may thus be concluded from this series of experiments that when diphteria toxin was injected at a later period after hypophysectomy (on the 11th-12th day), although it weakened to some degree the formation of structural changes, the operation did not prevent them from developing. It may also be suggested from the results obtained that the hormonal influences of the anterior lobe of the hypophysis are not the sole factor regulating the function of the adrenal cortex.

It will be clear from the experiments described that removal of the hypophysis alters the tissue reactivity. We therefore considered that it would be interesting to investigate the influence of hypophysectomy on immunogenesis. The few facts reported in the literature are contradictory [2, 4].

We carried out two series of experiments on 34 guinea pigs. In both series of experiments adsorbed diphtheria toxoid, preliminarily diluted 1:3 in physiological saline, was injected subcutaneously into the right hindlimb of the animals, in a dose of 21 antitoxin units, 5-6 days after hypophysectomy. The content of antitoxin in the serum from blood extracted from the heart was determined on the 10th-12th, 21st, and 42nd-44th day after immunization. The blood was investigated on the 10th-12th, 21st, and 28th day after revaccination, which was carried out on the 44th-46th day.

In the first series of experiments toxoid was injected a second time in the same dose and into the same place as in the first injection. The results of the investigations show that the antibody titer in both groups of animals rose slowly, starting on the 21st day after primary immunization. A sharp rise of antitoxin titer was observed after reimmunization on the tenth day, and an even greater increase on the 21st day. We found no clear difference between the antitoxin contents of the sera of the experimental and control animals. The results obtained are shown in Table 1.

In the second series of experiments, in contrast to the first, when reimmunization was carried out only half the dose of diphtheria toxoid was used (10.5 antitoxin units); the remaining experimental conditions were the same as in the first series. The results are given in Table 2.

TABLE 2. Antitoxin Content (in Antitoxin Units) in the Blood of Guinea Pigs at Various Times of Immunization with Diphtheria Toxoid in Hypophysectomized and Intact Animals (Second Series of Experiments; M ± m)

Group of animals	After first injection			After second injection	
	10th day	21st day	44nd day	10th day	28th day
Experimental	0,003 (0,002±0,011)	0,050 (0,054±0,009)	0,635 (0,633±0,066)	1,750 (1,875±0,125)	7,200 (7,170±0,166)
Control	0,007 (0,006±0,001)	0,062 (0,062±0,012)	0,567 (0,567±0,034)	1,580 (2,429±0,029)	6,800 (7,107±0,612)

The experiments thus showed that hypophysectomy, when performed 5-6 days before the beginning of immunization, has no significant effect on the course of the immunological reactions.

To summarize our findings, we may conclude that hypophysectomy alters the tissue reactivity of the body in relation to the action of diphtheria toxin. This was shown in our experiments, in particular, by the severity of the structural changes in various organs and tissues (the adrenal cortex, the myocardium, etc.), which are characteristic of the action of diphtheria toxin.

The modified reactivity of hypophysectomized animals in response to injection of diphtheria toxin may return to normal in the course of time; this suggests that the exclusion of the function of the hypophysis may be compensated to some extent by extrahypophyseal processes. This is also suggested by the absence of marked changes in the processes of immunogenesis after preliminary hypophysectomy. The results of our experiments show that although the stress reactions are of some importance in the mechanisms of development of diphtheria toxicosis, they do not determine the whole character of reactivity of the body to a pathogenic stimulus. The reactivity of the body is the resultant of many functions, among which an important role belongs to the nervous system a fact which was demonstrated by numerous investigations conducted by A. D. Speranskii's school.

SUMMARY

An inquiry was made into the effect of hypophysectomy on the formation of the structural changes in the internal organs of experimental animals following administration of diphtheria toxin at remote postoperative periods, as well as on the development of the specific diphtheria immunity. Removal of hypophysis 11 to 12 days prior to the diphtheria toxin injection has somewhat reduced the degree of affection, without preventing the development of the morphological changes in the organs, as noted earlier in cases with the toxin administered 3-4 days after the operation. The changes in the organs of the experimental animals,with the toxin administered on the 11th-12th day after hypophysectomy,were of the same nature as in controls, but less frequent and less intensive. As shown experimentally, the removal of hypophysis 5 to 6 days prior to the administration of sorbed diphtheria toxoid had no significant effect on the course of the immunological reaction.

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