ADAPTATION TO ESTROGEN OVERDOSAGE

An Acquired Hormone Resistance Without Antihormone Formation

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The fact that an animal may become resistant to the action of a hormone after a period of pretreatment has first clearly been shown for the parathyroid hormone (1). Later we made similar observations concerning the gonadotropic principles and concluded that in the latter case the resistance is due to the formation of hormone antagonistic substances since we found that "the blood of animals which have become insensitive to the anterior pituitary-like hormone of pregnancy urine is able to inactivate this hormone" (2, 3). On the basis of these experiments, Collip (4) formulated the theory that resistance to gonadotropic hormone develops because the organism produces a specific "antihormone" against this principle. Numerous other instances in which the antihormone theory of hormone resistance proved applicable have recently been considered in our detailed review of this subject (5). It was observed, however, that in many cases, hormone resistance may develop without the formation of demonstrable antihormones. Thus we consistently failed to reveal any parathyroid hormone neutralizing effect in the blood of animals pretreated with this principle (6). Similarly we showed that although chronic treatment with adrenalin fails to elicit the formation of a specific adrenalin antagonizing principle, it gives the organism a very marked resistance against this hormone (7, 8). We noted, furthermore that the enlargement of the pituitary, the adrenal cortex and the corpora lutea elicited in the rat by estrogen overdosage regresses after several weeks of treatment in spite of daily estrogen administration. We had to conclude therefore, that the organism may acquire estrogen resistance (9) although others have shown (10–12) and we may confirm (6) that estrin does not lead to antihormone formation. More recently we noted that young rats chronically treated with large doses of estrogens show a marked decrease in body weight during the first week but then reveal the development of resistance by the resumption of normal growth and weight increase. This readily distinguishable external sign of adaptation to the systemic effects of estrogen overdosage proved to be a useful indicator which served

as a basis for the experiments to be reported in this communication. The main purpose of these experiments was to analyze further the phenomenon of hormone resistance without antihormone formation and to establish the degree of specificity of such a resistance. As will be seen from the experiments reported in the experimental part of this paper, this resistance is not very specific inasmuch as pretreatment with estradiol renders animals resistant to the artificial synthetic estrogen diethylstilbestrol and *vice versa.*

EXPERIMENTAL PART. In our first experiment, we used 32 male and 32 female growing albino rats. At the beginning of the experiment the body weight of the males varied between 110 and 143 grams and that of the females between 107 and 145 grams. Eight males and 8 females received daily doses of 2 mgm. of estradiol subcutaneously in 0.1 cc. of peanut oil while 8 males and 8 females were given subcutaneous injections of the same

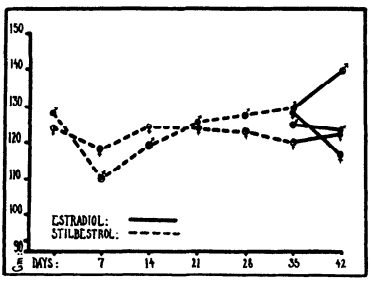

Fig. 1

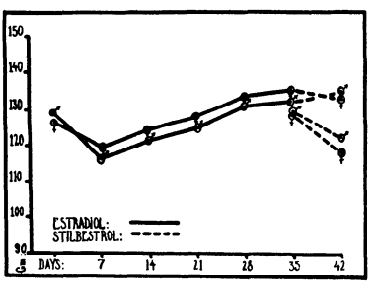

Fig. 2

Fig. 1. Weight curves of rats adapted to a large dose of stilbestrol, then treated with estradiol and of controls not adapted receiving estradiol during the last week of the experiment.

Fig. 2. Weight curves of rats adapted to a large dose of estradiol, then treated with stilbestrol and of controls not adapted receiving stilbestrol during the last week of the experiment.

daily dose of 4:4′-dihydroxy-α:β-diethylstilbene (diethylstilbestrol or stilbestrol) dissolved in the same amount of peanut oil. The animals were weighed at the onset of the experiment and then once a week throughout the treatment period. From their average weight, we constructed the growth curves shown in figures 1 and 2. These graphs indicate that during the first week, the rats of both sexes lost a great deal of weight as a result of the estrin treatment. By the end of the second week their weight had begun to increase, however, and in spite of continued daily treatment with the estrogens, the animals in all groups except in that of the diethylstilbestrol treated females continued to gain until the fifth week. In the latter group, after the recovery from the initial weight loss, the body weight remained fairly constant. It is noteworthy that although before treatment, the males of both experimental series were larger than

the females, the former lost so much more weight during the initial phase of estrogen administration that by the end of the first week, their average weight was below that of the females. At the end of the fifth week, the treatment of the animals adapted to estradiol was changed to diethylstilbestrol, while the animals which had so far been treated with the latter compound were given the same amount of estradiol for one week. As the graph indicates, this change in the type of the estrogen administered did not lead to any significant difference in the growth rate. At the same time, that is to say, during the fifth week of the experiment two other groups each consisting of 8 male and 8 female rats—which had not been pretreated but were approximately comparable in size to the estrogen pretreated animals—were given similar daily injections of estradiol and diethylstilbestrol respectively. The graph shows the considerable loss of weight which resulted from this treatment, a loss of weight which is approximately similar to that occasioned by the estrogens in the pretreated group during the first week of treatment. This experiment indicates quite clearly that resistance may be acquired to the toxic actions of both estradiol and diethylstilbestrol and that pretreatment with one of these estrogens renders the animals resistant to both estrogenic compounds.

It appeared of interest to establish whether a similar resistance to the toxic effect of large doses of estrogen may also be obtained by pretreatment with smaller doses. In order to determine this and to obtain further evidence supporting the conception of "crossed resistance" between the naturally occurring and artificial estrogens, we performed the following experiments.

Sixteen male (body weight 100–129 grams) and 16 female rats (body weight 96–120 grams) were divided into two groups, each of which contained 8 males and 8 females. One group received daily injections of 300 gamma of diethylstilbestrol and the other 300 gamma of estradiol subcutaneously in 0.1 cc. of peanut oil daily. Figures 3 and 4 show that this dose was tolerated without any loss of weight by the diethylstilbestrol treated males and the estradiol treated females while the remaining two groups showed a slight initial decrease in the average body weight. However growth was soon resumed by all groups and at the end of the fourth week, the body weight was far above the initial value in all animals. At this time, the estradiol pretreated rats were changed to daily injections of 1 mgm. of diethylstilbestrol while the animals pretreated with the latter substance received 1 mgm. of estradiol. Both substances were administered subcutaneously in 0.1 cc. of peanut oil. As indicated by our graphs, the trend of the growth curves was not significantly altered by this change to a large dose of a different estrogen. At the end of the fourth week, that is to say, at the time when the change in treatment occurred, we

began injecting a group of 8 males and 8 females with similar 1 mgm. daily doses of diethylstilbestrol and another group of 8 males and 8 females with the same amount of estradiol. These animals which had not been pretreated with estrogens showed a considerable decrease in body weight as indicated in figures 3 and 4, thus demonstrating that the pretreated group has really acquired a definite estrogen insensitivity.

Discussion. Our experiments indicate that the organism may adapt itself at least to the toxic effects of both estradiol and stilbestrol. While this is merely a confirmation and extension of previous observations (9, 13–15), it is of particular interest that adaptation to the naturally occurring steroid hormone, estradiol, induces resistance to a stilbene derivative which has a different chemical structure. At least in this instance, it appears that acquired hormone resistance without antihormone

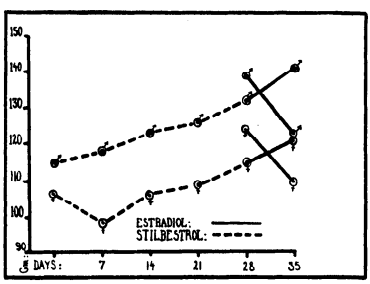

Fig. 3

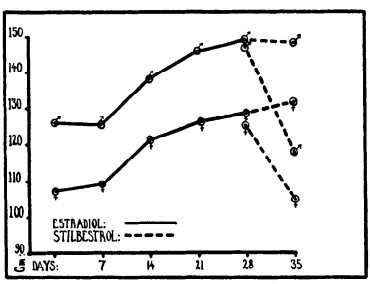

Fig. 4

Fig. 3. Weight curves of rats adapted to a relatively small dose of stilbestrol, then treated with a large dose of estradiol and of controls not adapted receiving estradiol during the last week of the experiment.

Fig. 4. Weight curves of rats adapted to a relatively small dose of estradiol, then treated with a large dose of stilbestrol and of controls not adapted receiving stilbestrol during the last week of the experiment.

formation is less specific than it usually is in cases of antihormone production. It will be recalled that resistance to gonadotropic preparations from the pituitary which is due to antihormone formation does not render animals insensitive to gonadotropic pregnancy urine extracts or *vice versa* (2, 3). Furthermore, antihormones elicited by treatment with a certain thyrotropic preparation have little or no effect on thyrotropic extracts prepared by a different method (16, 17).

With regard to the theoretical interpretation of the mechanism responsible for the crossed resistance between the two estrogens which we used, the following two possibilities present themselves. First it is conceivable that diethylstilbestrol is transformed in the organism to a substance similar to or identical in structure with estradiol and that it acts only after transformation into a compound of the steroid type. If this were

true, we would not be dealing with a real example of crossed resistance, since at the time when the estrogens which we employed exerted their pharmacological action, they would have become chemically identical or at least extremely similar. However, this interpretation receives no support from the bioassays which demonstrate that weight for weight, diethylstilbestrol is more active than most of the natural estrogens. The findings of Stroud (18) likewise indicate that the assumption of such a transformation is unfounded since he showed that diethylstilbestrol may be recovered as such from the urine of rabbits treated with this compound. It is true that the recovery is not quantitative but the author states that ". . . 4:4′-dihydroxy-α:β-diethylstilbene give recoveries of the order of 20 per cent compared with 1.5 per cent found for oestrone. This indicates a metabolic process for the synthetic oestrogens different from that of oestrone." These observations are not readily compatible with the view that diethylstilbestrol acts only after conversion into a steroid estrogen similar to the naturally occurring compounds.

A second and more likely explanation of our findings is that the organism does not become resistant to a certain chemical substance with estrogenic actions (that is to say, in our case to estradiol or diethylstilbestrol) as such, but to the estrogenic effect itself. If such an interpretation should prove to be correct, this case of crossed resistance would deserve special interest inasmuch as it would be a case of *adaptation to an action or effect rather than to a certain chemical substance or drug.*

Before closing this discussion, we should like to add that in numerous experimental series we noted that with very long continued estrone, estradiol or diethylstilbestrol treatment in doses of 2 to 5 mgm. daily, rats weighing approximately 100–150 grams at the onset of the experiment show the usual loss of weight during the first week followed by adaptation and increase in weight during the subsequent 5 to 6 weeks which is in accord with what has been said above. In these very chronic experiments however, where treatment was continued for 2 to 3 months, we invariably observed that after the period of adaptation, a third stage follows during which the acquired resistance appears to vanish so that the animals begin to lose weight again just as they did before adaptation occurred. We are not reporting these experiments in detail mainly because, unlike the initial period of weight loss and the period of adaptation, the final exhaustion of the adaptive mechanism and loss of weight is subject to great individual variations, one animal of a group beginning to lose weight after 5 or 6 weeks while another may go on for three months before its weight curve declines. Because of this great variability, each animal would have to be considered separately and since little would be gained from the study of such long individual weight curves or tables, we are omitting them here for the sake of brevity. Yet we want to mention these observations because they confirm our contention that the syndrome of adaptation to

estrogens is similar to that which develops during adaptation to various other damaging agents. It has been shown that during adaptation to almost any change in the internal or external environment of the organism, a so-called "general adaptation syndrome" is elicited which has three distinct stages. These have been termed the "stage of the alarm reaction," the "stage of resistance" and the "stage of exhaustion" respectively. During the first stage, among other symptoms and signs there is considerable loss of body weight, adrenal enlargement, involution of the lymphatic organs and water retention. During the second stage, most of these changes disappear and the animals become very resistant to the agent with which they are treated. Eventually however, during the third stage they again begin to lose weight and finally die with symptoms and signs similar to those seen during the initial stage. On the basis of these observations, we assumed that there is a general non-specific adaptation mechanism which facilitates the acquisition of resistance against a great many agents and that in case of prolonged exposure, this mechanism wears out and the adaptability or "adaptation energy" becomes exhausted (8). The literature on this adaptation syndrome has recently been reviewed by Leblond (18) and Varangot (19). Selye et al. (20) and Selye (21) showed that acute overdosage with estrogens may lead to a typical "alarm reaction" and the present experiments indicate that under the influence of continued treatment with estrogenic substances a "stage of resistance" and finally a "stage of exhaustion" develops. From this we may conclude that even adaptation to estrogens is not permanent but subject to the general law of the eventual exhaustion of adaptation energy as outlined in the above mentioned publications.

SUMMARY

Experiments indicate that the weight of growing rats chronically treated with estrogens first declines but growth is later resumed and the body weight may actually exceed the initial level in spite of continued administration of massive doses of estradiol or diethylstilbestrol. This is interpreted as additional evidence indicating that adaptation is possible at least to the toxic action of these estrogens although they do not elicit antihormone formation.

Rats adapted to the natural estrogen, estradiol, prove to be equally resistant to the artificial estrogenic compound, diethylstilbestrol, although chemically the latter is quite different from the former. Conversely diethylstilbestrol pretreatment renders the rat resistant to the toxic actions of estradiol. A review of the literature gives no support to the assumption that this "crossed resistance" should be regarded as due to the transformation of diethylstilbestrol into a naturally occurring estrogen similar to or identical with estradiol. It is concluded therefore, that the most probable interpretation of these findings is that we are dealing with a case of

acquired resistance to a certain (estrogenic) pharmacological action rather than to a particular chemical substance.

In case of very prolonged daily administration of relatively large doses of estrogens, the acquired adaptation is gradually lost. The animals, which following an initial stage of weight loss had adapted themselves to the treatment sufficiently to gain weight in spite of continued treatment, eventually lose weight again and finally die after two or three months of estrogen administration. It is concluded that adaptation to the toxic actions of estrogens takes place in the same manner as adaptation to most noxious agents, namely, by the development of the "general adaptation syndrome" with its characteristic three stages. *The eventual loss of an already acquired adaptation to estrogens gives further support to the conception that the adaptability or "adaptation energy" of the organism is a limited quantity and is gradually consumed while the organism puts up resistance against a stimulus to which it appears to be adapted.*

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REFERENCES

(1) SELYE, H. Endocrinology **16:** 547, 1932.
(2) SELYE, H., J. B. COLLIP AND D. L. THOMSON. Proc. Soc. exper. Biol. and Med. **31:** 487, 1934.
(3) SELYE, H., J. B. COLLIP AND D. L. THOMSON. Proc. Soc. exper. Biol. and Med. **31:** 566, 1934.
(4) COLLIP, J. B. J. Mount Sinai Hosp. **1:** 28, 1934.
(5) COLLIP, J. B., H. SELYE AND D. L. THOMSON. Biol. Reviews, **15:** 1, 1940.
(6) SELYE, H. (unpublished)
(7) SELYE, H. This Journal **122:** 347, 1938.
(8) SELYE, H. This Journal **123:** 758, 1938.
(9) SELYE, H., J. B. COLLIP AND D. L. THOMSON. Proc. Soc. exper. Biol. and Med. **32:** 1377, 1935.
(10) D'AMOUR, F. E., C. DUMONT AND R. G. GUSTAVSON. Proc. Soc. exper. Biol. and Med. **32:** 192, 1934.
(11) TWOMBLY, G. H. Endocrinology **20:** 311, 1936.
(12) SSARGIN, K. D. AND I. A. PLETENEVA. Bull. Biol. e. Med. exper. USSR **3:** 455, 1937.
(13) NOBLE, R. L. J. Endocrinol. **1:** 128, 1939.
(14) MACDONALD, I. G. Surgery **63:** 138, 1936.
(15) EMMENS, C. W. J. Physiol. **95:** 379, 1939.
(16) WERNER, S. C. Proc. Soc. exper. Biol. and Med. **34:** 392, 1936.
(17) WERNER, S. C. Proc. Soc. exper. Biol. and Med. **34:** 390, 1936.
(18) LEBLOND, C. P. Ann. d'Endocrinol. **1:** 179, 1939.
(19) VARANGOT, J. La Presse Med. **48:** 103, 1940.
(20) SELYE, H., C. M. HARLOW UND J. B. COLLIP. Endokrinol. **18:** 81, 1936.
(21) SELYE, H. Fondation Singer-Polignac Sex Hormone Conference, Paris 1937.