Evaluation of Tetrazolium as a Histochemical Index of Adrenal Cortical Activity.* (18519)

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In recent years, a number of specialized histochemical technics have been developed (1-4) in an effort to analyze the functional activity of the adrenal cortex more specifically than conventional procedures permit. These new tools provided an abundance of information enabling us to relate the adrenal cortex to different physiological and pathological processes. They were, however, limited in their scope, chiefly because of the static nature of the indices employed. It has proved difficult to measure objectively the dynamic processes of cell secretory activity, solely on the basis of criteria such as the intensity and character of the lipid distribution, or total cholesterol and ascorbic acid content. What appears to be lacking is some additional parameter which would provide a direct measure of the metabolic activity of the adrenal cortex and thereby enable us to interpret histochemical data more adequately. Unfortunately, the use of microrespiratory studies has as yet provided little or no metabolic evidence of this character. Other studies in our laboratory(5) led us to an exploration of the potential usefulness of 2,3,5-triphenyl tetrazolium chloride (TTC) for this problem. This reagent was selected because of a number of interesting properties. TTC is a colorless, water soluble compound which is transformed into a red, insoluble formazan by an active reduction process. In viable tissue slices, this chemical reaction involves an enzymatic reduction by intracellular reductases, such as the dehydrogenases, for which TTC acts as a hydrogen acceptor. The intracellular deposits of the colored formazan are clearly identified in frozen sections made after the tissues have been incubated in a TTC solution.

These properties of TTC have certain potential advantages with respect to the problem of adrenal cortical secretory activity. The reaction with TTC is carried out *in vitro* with viable slices of tissue and hence reflects the metabolic activity of the tissue at the time of study. The recent work of Kun and Abood(6), Antopol, Glaubach and Goldman (7), Ruttenberg, Gofstein and Seligman(8), Black and Kleiner(9), has demonstrated the validity of the reduction of TTC by living systems as a reflection of the metabolic activity of different cells. It is not yet known whether the reduction of TTC is an index of the activity of a specific dehydrogenase system or is an overall measure of different reductases. There is a growing body of evidence from various tissues that the extent of the deposition of the reduced TTC is roughly proportional to overall metabolic activity (unpublished observations). A more detailed analysis of the metabolic activity of a tissue, relating TTC reduction to specific enzymatic

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1. Dempsey, E. W., in *Recent Progress in Hormone Research*, 1948, v3, 127.

2. Greep, R. O., and Deane, H. W., *Ann. N. Y. Acad. Sc.*, 1949, v50, 509.

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5. Zweifach, B. W., Black, M. M., and Shorr, E., Proc. Soc. Exp. Biol. and Med., 1950, v14, 848.

6. Kun, E., and Abood, L. G., *Science*, 1949, v109, 144.

7. Antopol, W., Glaubach, S., and Goldman, L., *Trans. N. Y. Acad. Sci.*, 1950, Series II, v12, 156.

8. Ruttenberg, A. M., Gofstein, B. S., and Seligman, A. M., *Cancer Research*, 1950, v10, 113.

9. Black, M. M., and Kleiner, I. S., *Science*, 1949, v110, 660.

systems, is made possible by varying the incubation medium by the addition of different substrates or selective enzyme inhibitors. It is hoped that the TTC procedure, when utilized in this fashion, may prove to be a valuable adjunct to the histochemical procedures at present available for evaluating the functional activity of the adrenal cortex. The present report is concerned with our initial application of the TTC technic to this problem.

Methods. Tissues were obtained from rats after stunning the animals by a blow on the head, and from dogs at operation under pentobarbital anesthesia. The organs were rapidly transferred into ice-cold saline, after which slices 1.0 to 2.0 mm thick were cut with a razor by hand, as for the conventional Warburg microrespiration studies. Special precautions were taken to cut the slices at right angles to the surface in order to include all 3 zones in each section. The tissue slices were briefly rinsed in chilled saline and placed in a 25 cc Erlenmeyer flask containing 5 cc of the incubation medium. This consisted of 3 parts of a 1% solution of 2,3,5-triphenyl tetrazolium chloride (TTC) to one part of physiological saline, the mixture being brought to pH 7.2 with 0.1 M PO_4 buffer. In order to provide an excess of TTC, a maximum of 2 to 4 slices (approximately 200 mg) of tissue to 5 cc of the incubation medium was routinely used. The flasks were covered with parafilm to prevent evaporation and gently shaken in a Warburg bath at 37.5°C for one hour in air. At the end of this period, the solution was decanted and the stained tissue fixed in 10% neutralized formalin. Exposure to direct sunlight was avoided so as to minimize its effect on the formazan pattern. For microscopic study, frozen sections varying from 5 to 15 μ in thickness were cut and mounted in glycerin. Duplicate sections were routinely counterstained with polychrome methylene blue, to allow better histological and cytological identification. Preparations stained with TTC gradually lose their color after repeated exposure to light and should be kept in the dark. In the present study, the sections were examined immediately after preparation and photographs (black and white, and kodachrome) were routinely taken within 24 hours after the sections were prepared. In selected experiments, the reduction of TTC was carried out in the presence of an added substrate, such as succinate, in order to accentuate the deposition of the formazan (3 parts 1% TTC—PO_4 buffer mixture to 1 part 0.2 M succinate). The histochemical intracellular staining pattern under these conditions was otherwise identical with that obtained in the absence of added substrate. However, an accentuation of extracellular deposition of formazan was noted, particularly in the junctional area between the glomerulosa and fasciculata.

Experimental results. 1. *Normal adrenals.* Following *in vitro* incubation with TTC, tissue slices become diffusely stained. An essentially similar type of diffuse staining was observed in the adrenals of normal mice, rats, guinea pigs, rabbits and dogs. Quantitative determinations indicated that the intensity of the TTC reduction by the adrenal would place it among the more active tissues in this respect. In terms of gamma TTC reduced/mg acetone dried tissue, the mean values for slices of dog kidney and adrenal are 4.2 and 1.7 γ/mg, respectively. The reduced TTC does not appear as discrete granules or crystals in the cytoplasm of the cell as it does in the kidney. Instead, the formazan is exclusively located in the lipid droplets of the cytoplasm, presumably because of its lipid solubility.

In the rat, the deposition was of uniform intensity throughout the 3 zones (Fig. 1A). In the dog, guinea pig and man, an especially prominent deposit of reduced TTC usually occurred in the zona glomerulosa. With conventional technics, there is no histologic distinction between the glomerulosa cells and the cells of the fasciculata where they lie directly adjacent to one another. However, with TTC, cells in the glomerulosa were readily identified since their lipid droplets were larger and were stained a more intense red than were those of the adjacent fasciculata (Fig. 1B, 1C), a further indication of a functional difference between these two zones. In general, it appeared that the cells of the reticularis were stained a more intense red than the inner fasciculata cells. In addition,

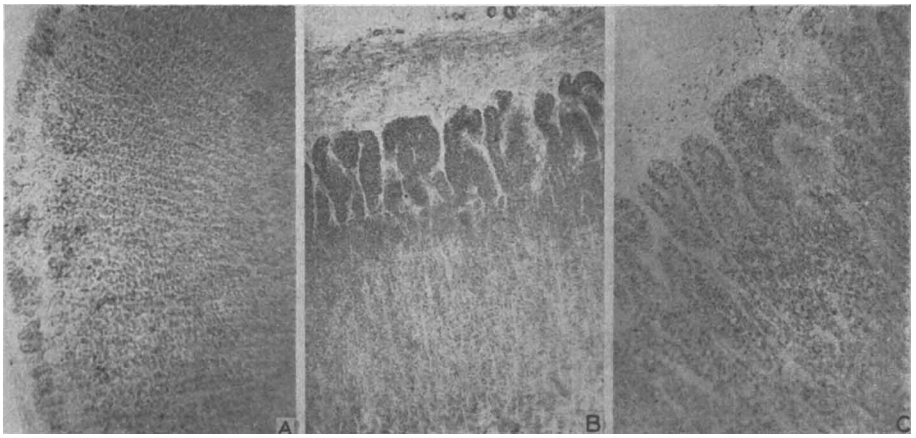

FIG. 1A. Adrenal cortex of normal rat after incubation with TTC for 1 hr. Note pale zone intermediate between glomerulosa and fasciculata in which no TTC deposition occurred. (Magnification 24×).

FIG. 1B. Adrenal cortex of normal dog stained with reduced TTC. The glomerulosa is prominent. (24×).

FIG. 1C. Adrenal cortex of normal dog under higher magnification (40×), showing distribution of reduced TTC within lipid granules of cells.

the lipid in these cells was usually more coarsely dispersed. Isolated cells, with staining characteristics similar to those of the reticularis cells, were often seen in the adrenal medulla, although direct anatomical connection with the cortex proper could not be seen. The cells of the adrenal medulla proper showed no significant intracellular deposition of formazan. There was, however, a variable amount of extracellular deposition of elongated and granular formazan crystals, as well as some purplish plaques throughout the medulla.

As can be seen in the accompanying photograph (Fig. 1A), the reduction of TTC in adrenals used for illustration was not always completely uniform, some areas being more heavily stained than others. The possibility of some of these variations being artifacts cannot be excluded at present. The staining of the fasciculata usually occurred fairly evenly, although in many sections there appeared to be a central zone in which the reduced TTC was sparse. This finding, however, was not a constant feature. These illustrations were selected to call attention to the occasional variations which were encountered.

Not infrequently, in tissues incubated with no added substrate, there was seen between the glomerulosa and the fasciculata of the rat adrenal a thin layer, several cells in thickness, which did not stain with formazan (Fig. 1A). While the cells of the glomerulosa characteristically have heavy lipid granules within the cytoplasm, the cells of the immediately adjacent zone contained little or no lipid (Fig. 1A). This intermediate TTC negative zone became more prominent under different experimental conditions, and, as will be shown later, was especially evident in the adrenals of hypophysectomized rats. In many sections of adrenal tissue there occurred a considerable deposit of reduced TTC extracellularly in the region of the sinusoids, the formazan appearing as coarse purplish plaques. This type of TTC reduction is most prominent in the glomerulosa and in the outer fasciculata. The endothelial cells lining the sinusoids of normal adrenals showed no intracellular TTC staining.

II. *Depleted adrenals.* A study was made of the tetrazolium reaction of the adrenal gland following two types of stress and during the acute response to the injection of ACTH. Six rats were each given 20 γ of ACTH† (with activity 8 times that of the Armour Standard)

† The ACTH used in these experiments was made available through the kindness of Dr. John R. Mote of Armour and Co., Chicago.

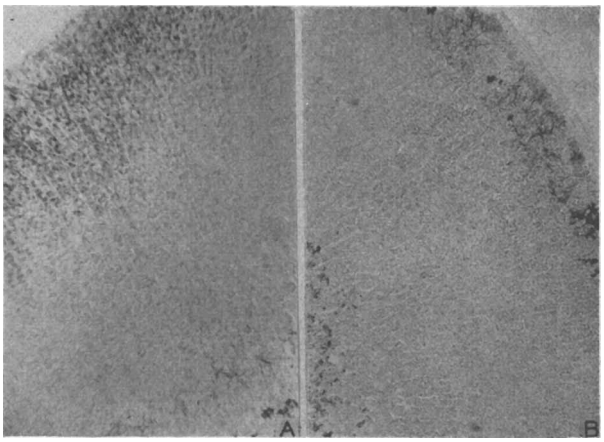

FIG. 2A. Adrenal cortex of rat stained with reduced TTC following the injection of 20 γ ACTH twice daily for 1½ days. The reticularis and inner fasciculata show pronounced depletion of lipid and are no longer stained with TTC. The formazan staining of the glomerulosa is unaffected. The unstained zone between the glomerulosa and fasciculata is not visible. (24×).

FIG. 2B. Adrenal cortex of rat stained with formazan following hemorrhagic shock for 2 hr. All three zones show a diminished capacity to reduce TTC, especially the fasciculata and reticularis. Some reductase activity is still present in the glomerulosa. (24×).

intramuscularly, twice daily. Three of the rats were sacrificed after 36 hours on this regime. Using TTC staining as a criterion, the most pronounced effect of the adrenocorticotrophic hormone was seen in the fasciculata. The cells of this zone showed an almost complete lack of TTC reduction, concomitant with a depletion of intracellular lipid. The cells of the reticularis continued to reduce TTC, with little or no reduction in their lipid content. The zona glomerulosa did not appear to be affected by the administration of this amount of ACTH (Fig. 2A).

Three rats were exposed to cold (1°C) for 72 hours and the adrenals immediately removed for *in vitro* study. In these animals, although the glomerulosa continued to stain, the two inner zones no longer reacted to TTC. The formazan staining indicated a marked falling off of reductase activity in both the fasciculata and reticularis. It is significant that the loss in TTC reduction was also accompanied by a depletion of the cytoplasmic lipid in the cells of the fasciculata and reticularis.

Several rats were subjected to hemorrhagic shock. The blood pressure was maintained at 40 mm Hg for 2 hours and the animals were then sacrificed. The adrenals in these rats showed the most profound reduction in formazan staining of the 3 stress situations employed here (Fig. 2B). The fasciculata and reticularis no longer reacted with TTC, and even the glomerulosa showed a significant reduction of its TTC activity. Here again, loss of the capacity to reduce TTC was paralleled by a loss of the preformed lipid from the cell.

III. *Adrenal atrophy.* (A) *Hypophysectomy.* Overall atrophy of the adrenal gland was brought about by hypophysectomy. A series of 12 rats, 30 to 40 days old, was studied at different stages following hypophysectomy.‡ These rats were allowed free access to both the stock rat pellets and the synthetic diet recommended by Shaw and Greep(10). They appeared well nourished

‡ We are indebted to the Hormone Assay Laboratories, Inc., Chicago, for the hypophysectomized animals used in the present study.

10. Shaw, J. H., and Greep, R. O., *Endocrinology,* 1949, v44, 520.

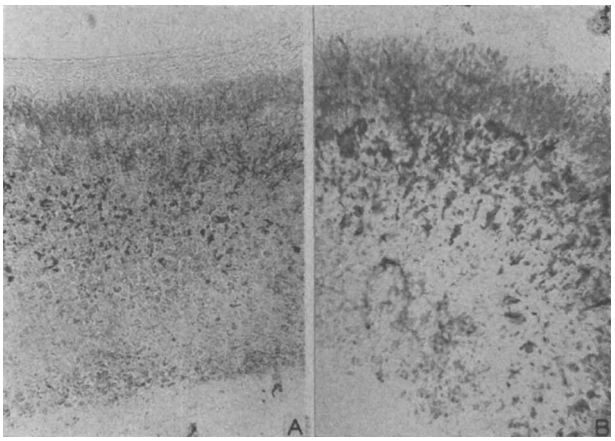

FIG. 3A. Adrenal cortex of rat stained with reduced TTC 11 days after hypophysectomy. The fasciculata and reticularis begin to show a lack of staining in many cells, despite the presence of lipid. The glomerulosa is unaffected. (24×).

FIG. 3B. Adrenal cortex of rat stained with reduced TTC 50 days after hypophysectomy. There is marked atrophy of the 2 inner zones, together with completely irregular and deficient TTC staining. Scattered reticularis cells continue to reduce TTC. The glomerulosa continues to reduce TTC actively. The irregular dark areas represent extracellular deposition of reduced TTC. (24×).

and gained weight throughout the study. The first set of adrenals was removed 8 days after hypophysectomy. At this stage, the glands as a whole had already undergone partial atrophy. There appeared between the glomerulosa and the fasciculata an unusually prominent zone in which there was no formazan staining and in which the cells contained only sparse lipid. The glomerulosa appeared normal in its TTC reaction, both in terms of the extent of staining and in the intensity of the formazan deposition. The fasciculata in these early hypophysectomized animals showed diminished, spotty staining. The reticularis appeared normal in regard to lipid content and continued to react with TTC as evidenced by its uniform staining.

A second set of animals was sacrificed 11 days postoperatively. In these adrenals the glomerulosa continued to stain well and actually appeared somewhat thicker than normal. The two inner cortical zones showed extremely irregular staining with formazan, despite the presence of lipid in many of these cells (Fig. 3A). Many of the fasciculata cells showed no staining with formazan. Others reduced TTC, but the intensity of staining varied widely in different areas, in contrast with the uniform staining of the normal fasciculata. Similarly, there was a marked variation in the lipid content of the cells of the fasciculata. A more uniform deposition of TTC was noted in the cortical cells immediately adjacent to the medulla. At 23 days after hypophysectomy, marked generalized atrophy of the two inner zones, the fasciculata and reticularis, was evident (Fig. 3B). The glomerulosa was no longer as prominent as it was at 11 days, but still continued to stain well with formazan. The intermediate zone between the glomerulosa and the fasciculata, which was readily discernible in the above 2 sets of hypophysectomized rats, was no longer visible. Actually, a clearly defined zona fasciculata could not be demonstrated by TTC. The innermost reticularis cells adjacent to the medulla continued to deposit appreciable amounts of TTC. In addition, considerable extracellular deposition of formazan now occurred, especially in the region immediately below the glomerulosa. At 50 days postoperatively, no uniform de-

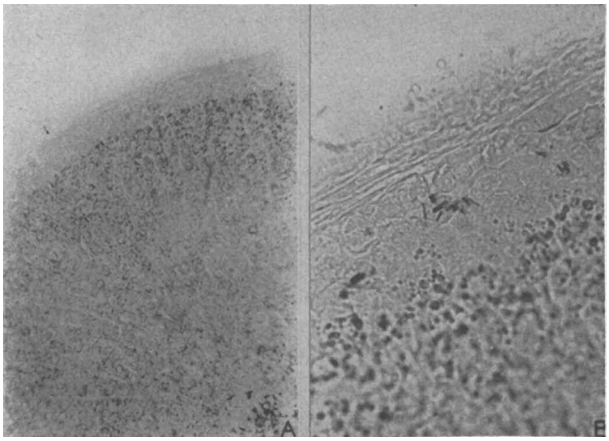

FIG. 4A. Effect of DCA on TTC staining of rat adrenal cortex. Complete suppression of reductase activity in the glomerulosa is evident, with no effect on the fasciculata and reticularis. (24×).

FIG. 4B. High power view showing TTC suppression limited to outer glomerulosa. (40×).

position of formazan occurred except in the zona glomerulosa. The reticularis continued to show scattered cells containing preformed lipid which was stained red with reduced TTC (Fig. 3B).

In sections which were counterstained with methylene blue there could be seen, interspersed throughout the two inner zones, irregularly shaped cells which did not stain with formazan but in which the cytoplasm was intensely basophilic. These cells were first noted as early as 8 days after hypophysectomy and could still be seen in adrenals removed 50 days after operation.

(B) *DCA inhibition of glomerulosa.* The usefulness of the TTC reaction as an index of functional activity of the adrenal cortical cells is best illustrated in experiments in which a particular zone of the adrenal is functionally depressed by the administration of a specific adrenal cortical hormone. Thus, a substance with mineralo-corticoid activity, such as desoxycorticosterone acetate, should, according to current concepts, selectively suppress the zona glomerulosa(11-13). Two 25 mg pellets of DCA were implanted subcutaneously in each of 12 rats. The rats were maintained on regular pellet rations and drinking water, and sacrificed after 14 to 30 days. As can be seen in the accompanying photograph (Fig. 4A), there was a specific effect on the zona glomerulosa, although no cytonecrosis was noted in the counterstained specimens. The cells showed a complete suppression of formazan staining, as well as a decrease of preformed lipid. The unstained cells of the glomerulosa were in sharp contrast to the contiguous cells of the fasciculata which continued to stain with formazan as under normal conditions (Fig. 4B). It is noteworthy that although some of the glomerulosa cells still contained preformed lipid, they did not reduce TTC. Work with specific enzyme inhibitors has shown that the enzymatic reduction of TTC may be inhibited without affecting the lipid content of the cell (unpublished observations).

IV. *Hypertrophy of the adrenal.* Hypertrophy was achieved by two procedures; first, by continued administration of 40 γ ACTH§ for 8 to 10 days, and second, by repeated sublethal trauma in the Noble-Collip drum. In both instances the adrenal gland underwent pronounced hypertrophy which was most evi-

11. Deane, H. W., Shaw, J. H., and Greep, R. O., *Endocrinology,* 1948, v43, 133.

12. Greep, R. O., and Deane, H. W., *Endocrinology,* 1947, v40, 417.

13. Selye, H., Stone, H., Timiras, P. S., and Schaffenburg, C., *Am. Heart J.,* 1949, v37, 1009.

§ Same preparation used previously.

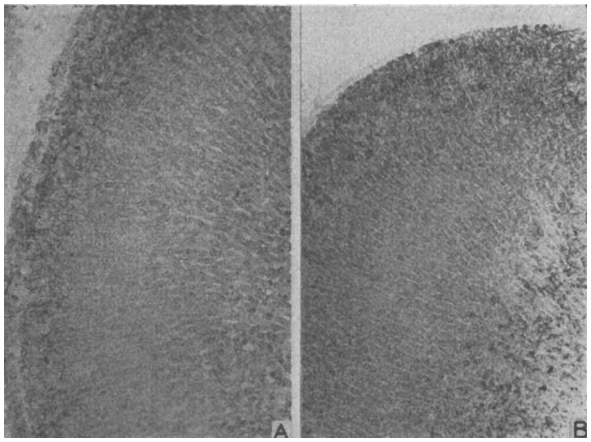

FIG. 5A. Hypertrophy of adrenal cortex of rat following chronic administration of ACTH. All 3 zones stain well with reduced TTC. Intermediate zone between glomerulosa and fasciculata still present. (24×).

FIG. 5B. Adrenal hypertrophy in rat subjected to repeated drum trauma. There is a considerable and uniform deposition of formazan throughout the gland. The intermediate unstained zone between glomerulosa and fasciculata is no longer demonstrable. (24×).

dent in the zona fasciculata (Fig. 5A, 5B). The cells in all 3 of the layers of the cortex contained considerable lipid. Following incubation with TTC, all of the cellular components uniformly reduced the TTC and were well stained. In adrenal hypertrophy, following ACTH administration, the unstained zone intermediate between the glomerulosa and the fasciculata was still visible. In adrenal hypertrophy following repeated sublethal trauma, the glomerulosa and fasciculata appeared fused so that distinction between the two zones was not possible on the basis of cytological features.

Discussion. It has been well established by previous workers(7-9) that the reduction of the colorless TTC compound to the insoluble red formazan, in tissue slice incubation studies of the type employed here, requires the enzymatic activity of living cells. It has also been found that the reaction is directly dependent upon temperature. Incubations carried out in air show a progressive reduction of TTC with time until a maximum is achieved after 60-90 minutes. The reduction is not carried out by injured or dead cells. Specific inhibitors which depress the metabolic activity of the cell also depress or completely abolish the ability of the cell to reduce TTC.

The application of this tool to studies of living cells was first made in experiments with seedlings(14) and then applied to a study of specific enzyme systems in unicellular organisms, such as yeast. Later, studies were carried out, incubating blocks of living tissue with TTC(15-17). It was not, however, until the work of Black and Kleiner(9) that use was made of the tissue slice technic as it is employed in microrespiration studies. These latter investigators(18) also found that the deposition of reduced TTC within the tissues could serve as a visible histochemical index to measure the activity of specific cells or cell components of different tissues. In a recent publication(5) from our laboratory, it was shown that TTC can be used as a histochemical index to detect metabolic alterations

14. Kuhn, R., *Ber. Deutsch. Chem. Ges.*, 1941, v74B, 949.

15. Mattson, M. A., Jensen, C. D., and Dutcher, R. H., *Science*, 1947, v106, 294.

16. Antopol, W., Glaubach, S., and Goldman, L., *Pub. Health Rep.*, 1948, v63, 1231.

17. Straus, F. G., Cheronis, N. D., and Straus, E., *Science*, 1948, v108, 113.

18. Black, M. M., Opler, S. R., and Speer, F. D., *Am. J. Path.*, 1950, v26, 1097.

in the renal proximal convoluted tubules of hypertensive patients and animals.

The capacity to reduce TTC under the aerobic conditions of these experiments (20% oxygen in air) is roughly proportional to the overall metabolic activity of the cell as determined by oxygen consumption measurements. Thus, when tissues are arranged in the order of their oxygen consumption, as measured by the conventional Warburg technic, their sequence is directly related to their capacity to reduce TTC. It should be emphasized that the TTC reaction is a measure of one or more hydrogen transfer mechanisms and therefore cannot be directly compared to oxygen consumption measurements which are a reflection of the total oxidative metabolism of the cell. It should also be recognized that cells probably carry out metabolic processes which do not involve reductase or dehydrogenase activities of the type measured by TTC. Changes in functional activity in these latter categories need not be reflected by changes in TTC reductase activity. The extent to which the reduction of TTC by the cells of the adrenal cortex is a reflection or a measure of the secretory activity of these cells is of primary interest. The secretory type of cell in the adrenal cortex contains numerous lipid granules of varying size. Such cells are seen in the normally functioning gland, as well as in the gland which has undergone hypertrophy. Cells which are hypofunctional, or undergoing atrophy, usually lose their lipid. We have found a definite parallelism between the deposition of formazan and the presence of lipid in the secretory type of cell. In normal and in hypertrophic adrenals, all of the zones of the cortex reduced TTC in a characteristic and uniform fashion. There were only two instances in which formazan staining did not occur, despite the presence of lipid in the cells. The first was seen in experiments with chemical inhibitors, where it was possible to obtain a specific suppression of TTC activity in the presence of intracellular lipid. Also, during the early stages of zonal inhibition following the injection of DCA, many of the cells in the glomerulosa were unable to reduce TTC, despite the presence of lipid granules. Since this hormonal effect apparently represents a condition in which the glomerulosa cells are undergoing atrophy and are functionally depressed, the absence of formazan staining is in accord with the concept of active TTC reduction as a reflection of the secretory activity of the cell.

Following hypophysectomy, a diminution or complete suppression of adrenal cortical secretory activity occurs in the fasciculata and reticularis of the rat(19,20). In our experiments, the development of a hypofunctional condition in these two zones was paralleled by a progressive failure of these cells to reduce TTC. The parallelism between adrenal cortical secretory function and the capacity to reduce TTC is thus again apparent.

Following acute stress or the discharge of the adrenal cortex by the administration of ACTH, a more complex situation exists. The cells of the fasciculata and reticularis discharge their lipid granules and, with conventional staining technics, appear depleted. This depleted state continues for a variable period of time before there is again clear-cut evidence of secretory activity as reflected by the appearance of intracellular lipid. The question remains as to what this morphological state represents in terms of functional activity. Is it a state in which the continued stimulation of the adrenal cortex results in a rapid and continuous discharge of the intracellular lipid secretions, so that no morphological evidence of such secretory activity is apparent, or does it represent a condition in which the cell is temporarily inactive and is slowly restoring its enzyme systems before it can again begin active secretion? The fact that these depleted cells do not actively reduce TTC would seem to favor the latter interpretation. This, however, does not exclude the possibility that the particular type of metabolic synthesis, in which the cells of the adrenal cortex are engaged at this stage, may not be reflected by the TTC reductase activity.

19. Deane, H. W., and Greep, R. O., *Am. J. Anat.*, 1946, v79, 117.

20. Selye, H., *J. Clin. Endocrinol.*, 1946, v6, 117.

The relative independence of the functional activity of the glomerulosa from that of the two inner zones of the cortex has been repeatedly demonstrated in the present TTC studies. Our data on adrenals of hypophysectomized rats indicate a continued staining of the glomerulosa, while the fasciculata and reticularis are losing this capacity. Likewise, the discharge of the fasciculata and reticularis by stress situations, such as cold, and by the administration of ACTH, is accompanied by no change in the capacity of the glomerulosa to reduce TTC. Finally, in experiments with DCA, the reduction of TTC by the glomerulosa was selectively suppressed, while the adjacent fasciculata and reticularis continued to stain. The demonstration of an intermediate zone between the glomerulosa and fasciculata was initially made by Sarason(21) and Simpson, Evans and Li(22), and also has been reported by Greep and Deane(2). We have been able to confirm the presence of such a zone in the rat adrenal with the TTC technic. This zone is clearly differentiated by its failure to reduce TTC. The precise significance of this finding is as yet unclear.

Mention has been made of the fact that the reduced TTC is regularly localized within the cytoplasmic lipid of the cells. This is in contrast to cells of other tissues which have no visible lipid granules in their cytoplasm and in which crystals of formazan are deposited as granules or needles. Furthermore, we have noted that active reduction of TTC is usually found in cells which contain lipid and which are in the process of active secretion. The question therefore arises as to the precise relationship of the TTC reductase activity and lipid formation. The lipid or lipo-protein complexes apparently do not possess the property of actively reducing TTC *per se*. It is our belief that the reduced formazan is localized in the lipid droplets because of its lipid solubility. Thus far, we have never observed, under normal conditions, adrenal cortical cells which are devoid of lipid and contain reduced TTC.

The TTC technic has permitted two types of evaluation of adrenal cortical activity. First, an overall estimation of the total amount of reduced TTC was made by photocolorimetric methods. These findings will be presented in a subsequent publication. Second, microscopic studies were made of the localization of formazan staining in specific zones of the adrenal cortex under various experimental conditions. At present, experiments are in progress with specific cortical hormones, such as Compounds B, E, F and S, in an attempt to determine their zones of origin. In addition, through the use of substrates and inhibitors, we are attempting to obtain more specific information concerning the precise enzyme systems which are related to the dehydrogenase activity of the cell as demonstrated by TTC reduction.

Summary. The application of the tetrazolium technic to a histochemical differentiation of the zonal activity of the various zones of the adrenal cortex is indicated. The functional activity of the cortical cells would appear to be paralleled by comparable changes in the ability of the cell to reduce tetrazolium. The implication of this type of approach to the problem of adrenal cytophysiology is discussed.

We are indebted to Mr. Jack Godrich for the microphotography in this study.

21. Sarason, E. L., *Arch. Path.*, 1943, v35, 373.

22. Simpson, M. E., Evans, H. M., and Li, C. H., *Endocrinology*, 1943, v33, 261.

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