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DISTRIBUTION OF SUCCINIC DEHYDROGENASE IN THE ORGANS OF THE ADULT ALBINO RAT

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The use of tetrazolium salts as hydrogen acceptors has made it possible to develop a histochemical technique by which areas of succinic dehydrogenase activity become intensely stained by the precipitation of formazanes [1, 2, 3]. The distribution of the enzyme was studied in various tissues and organs [4, 5, 6, 7, 8, 9, 10, 11, 12], its intracellular location [13], and its relative quantity [14, 15], as well as some functional variations and pathological changes [16, 17, 18, 19, 20, 21, 22, 23, 24]. The purpose of the present work was to study the distribution of the succinic dehydrogenase in various tissues and organs of the normal adult albino rat as an introduction to further experimental research or to the histochemical analysis of the enzyme during development.

Material and Technique

We used adult albino rats of both sexes weighing from 200 to 350 g. The animals were killed by a blow on the head and the organs rapidly removed and cut on the freezing microtome in sections of about 30 μ which were received directly into the incubation mixture and incubated at 37° C for half to one hour, according to the activity of the tissue. When the sections acquired the desired colour, they were washed quickly in distilled water and fixed in 10 % formaldehyde. They were then mounted in Apathy's gum syrup with or without previous staining of the nuclei.

The incubation mixture is prepared as follows:

From 1 to 2 g of potassium cyanide are dissolved in 50 cm^3 of distilled water with one drop of cresol red. Acetic acid is added until an orange shade is obtained and a buffer of phosphate M/5 pH 8 is added to a total of 100 cm^3. A separate solution

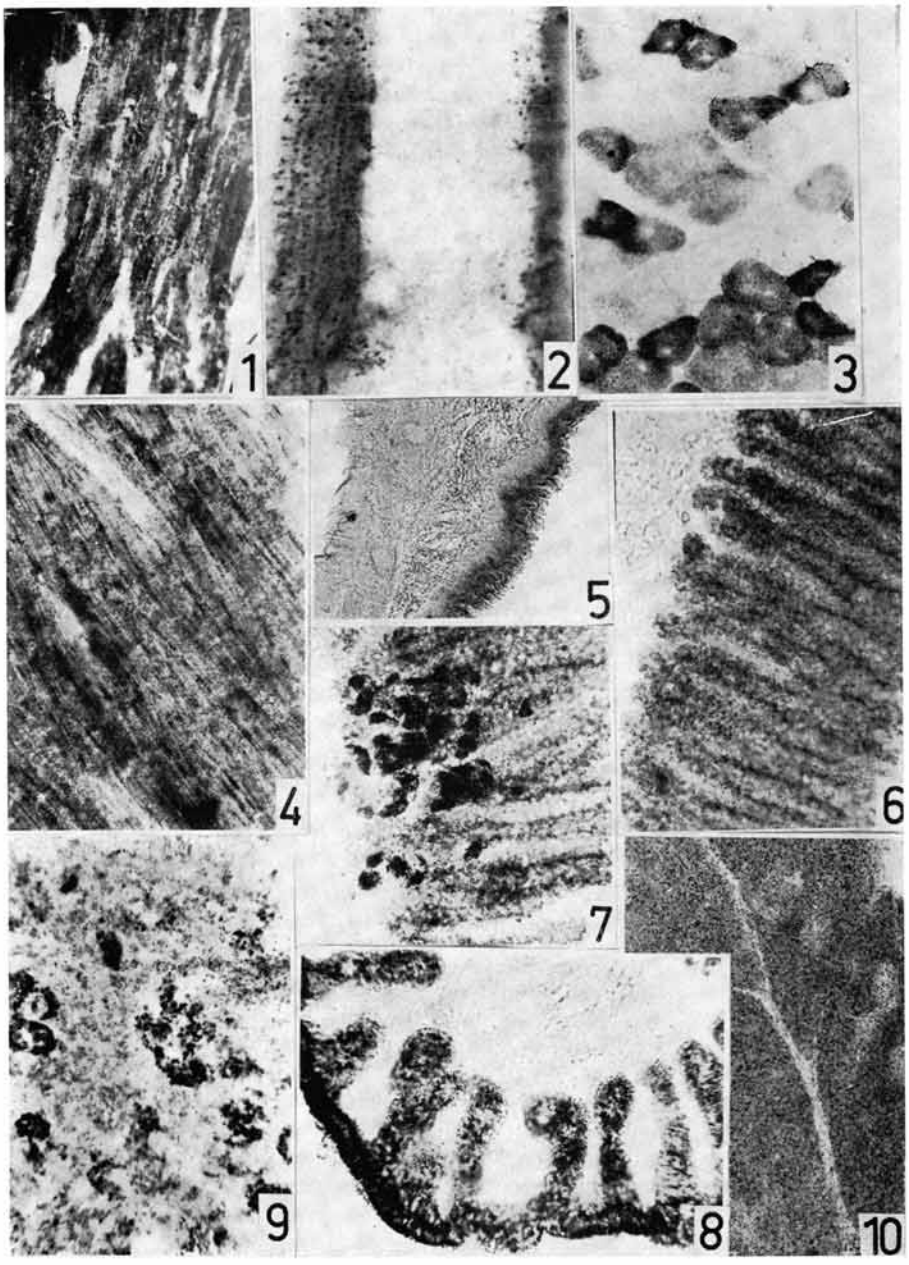

Fig. 1. Skeletal muscle (diaphragm). Longitudinal section. – *Fig. 2.* Skeletal muscle, showing a "white" fibre, with very low enzyme content, between two "red" fibres, rich in enzymes. – *Fig. 3.* Skeletal muscle. Cross section, showing the varying con-

is prepared of 0.25 mg of neotetrazolium chloride[1] in 5 cm^3 of 95 % alcohol, and then added to the former. Finally, 0.2 to 0.3 mg of sodium succinate is added.

The preparations are examined with an ordinary microscope or, in some instances, phase contrast, the better to determine the distribution of the reaction. We have classified the reaction into four grades, depending upon an approximate appraisal of the quantity of formazane formed; thus: weak, moderate, intense and very intense. We have also distinguished the blue granular reaction (due to the formation of diformazane) from the diffuse red reaction (due to monoformazane).

Results

Biochemical studies showed that succinic dehydrogenase is widely distributed in animal tissues, considerable quantities being found in the kidneys, liver and heart. The brain, testis, skeletal muscles, lungs, adrenals, spleen and retina had a moderate to weak activity. The thymus, pancreas and blood showed none whatever [25]. Nevertheless, as we shall see in the descriptions which follow, our findings permit us to state that a weak reaction existed in the pancreatic acini and in some structures of the spleen and thymus.

1. Muscles

a) Skeletal Muscles (figs. 1, 2, 3)

We have studied the gastrocnemius, the diaphragm, the muscles of the tongue, the masseter and the dorsal muscles. In all these the reaction varies from fibre to fibre. Some present an intense blue granular reaction with granules or very short rods arranged in chains regularly distributed between the myofibrils. In transverse sections of the fibres, the granules appear arranged regularly at the periphery thereof, leaving a central or slightly eccentric area free

[1] The neotetrazolium used was from Biotronics Laboratories, Coventry, Conn., and from Nutritional Biochemical Corporation, Cleveland, Ohio, the former having given us better preparations.

tent in the fibres, and the peripheral distribution. – *Fig. 4.* Heart muscle of the ventricle. – *Fig. 5.* Cardiac portion of stomach. Positive stratified squamous epithelium. – *Fig. 6.* Gastric mucosa of the fundus. Intense positive reaction, above all in the deep half of the glands. – *Fig. 7.* Glands of the fundic portion of the gastric mucosa. Parietal cells showing very intense reaction. – *Fig. 8.* Mucous coat of large intestine. Intensely positive *Lieberkühn* glands. Clear spaces correspond to goblet cells. – *Fig. 9.* Submandibular gland. – *Fig. 10.* Pancreas. Moderate granular reaction in the acini, and negative in the islets of *Langerhans.*

from the reaction (fig. 3). Neighbouring fibres may only show a weak reaction, or none at all (fig. 2). In general, slender fibres show a more intense reaction than do thick ones, and vice versa [4]. This is particularly evident in the masticatory muscles, in which the slender fibres predominate. Within the cell, the reaction is granular and confined to the myoplasm, among the myofibrils. The granules are arranged very regularly in long chains, which in some regions are reminiscent of the cross striations. The striated muscle of the tongue shows the same type of reaction we have described, but in this organ the varying intensity of the reaction from fibre to fibre does not exist, as it does in the gastrocnemius or in the diaphragm.

It is difficult to explain the very great difference in enzyme content which exists between adjacent cells. We believe that the slender fibres belong to the "red" type of fibre, while the thick fibres correspond to the "white" type [1, 26]. It appears that the "white" fibres, richer in myofibrils and poorer in sarcoplasm, contain more cytochrome than the "red" fibres [27].

b) Myocardium

In the muscle fibres of the heart, the reaction is very intense and granular, being similar to that of the skeletal muscles. However, all the fibres of the cardiac muscle show a similar degree and distribution of activity (fig. 4), constituting a marked difference from skeletal muscles, in which adjacent fibres vary considerably. On the other hand, differences have been found between the myocardial fibres of the ventricles and those of the atria, the former being richer in succinic dehydrogenase, a difference which becomes more marked with age [24]. In cases of myocardial infarction due to coronary occlusion, both experimental and in human beings, a rapid disappearance of the enzyme from the affected fibres was observed, being quite evident two hours after the first symptoms [23].

2. The Digestive System

The stratified squamous epithelium of the mucous membrane covering the tongue and oesophagus show rare granules situated in the cells of the basal layer.

In the stomach the reaction is granular, positive, and of a moderate degree in the stratified squamous epithelium lining the cardiac half of the stomach in these animals (fig. 5). Compared with the stratified squamous epithelia of all the other organs, this gives the most marked reaction, although it is poor.

The glandular portion of the stomach shows an intense reaction in the fundic glands. This reaction is most intense in the deep third of the gland, i.e., in the portion richest in parietal cells (fig. 6). The cytoplasm of these cells stains deeply, with irregular, coarse, granular formations which fill them completely (fig. 7). The zymogenic cells show a much weaker reaction, consisting of a few granules in the cytoplasm.

The smooth muscle of the stomach gives a weak granular reaction. In the pyloric region the glandular cells show an intense reaction near their apices. This reaction is more marked in the cells at the base of the glands than in those at the neck. The mucoid cells are almost devoid of granules.

The Intestine. There is an intense granular reaction in the cells of the crypts of *Lieberkühn*, seen both at the base and apex (fig. 8). The goblet cells are negative. The duodenal muscles have a marked granular reaction.

The Submandibular Gland. The reaction is finely granular in the small alveoli. It is coarse and intense in the clear ducts (fig. 9). The mast cells seen in this gland are negative.

The Parotid Gland. There is a fairly strong granular reaction in the alveoli and more intense granular reaction in the ducts.

The Pancreas. There is a positive granular reaction in the cells of the alveoli; the ducts and the islets of *Langerhans* are negative (fig. 10).

The Liver. There is a very intense reaction in the cytoplasm of the liver cells, but so distributed that it is much more so in the periportal cells. Thus, the hepatic lobules acquire a characteristic appearance, presenting a pale central portion, and a deeply stained periphery (fig. 11). The reaction evidently increases in normal rats injected with cortisone; it is weak in adrenalectomized animals, becoming normal once more when cortisone is administered (16]. *Kupffer* cells are negative. Within the hepatic cells the reaction is densely granular, there being also a diffuse staining of the fundamental cytoplasm, distinctive of this organ.

3. The Respiratory System

Larynx, trachea and bronchi. These show an intensely positive granular reaction in the epithelium and in the glands. The cartilage is completely negative (fig. 12).

Lungs. The pulmonary alveoli show no reaction.

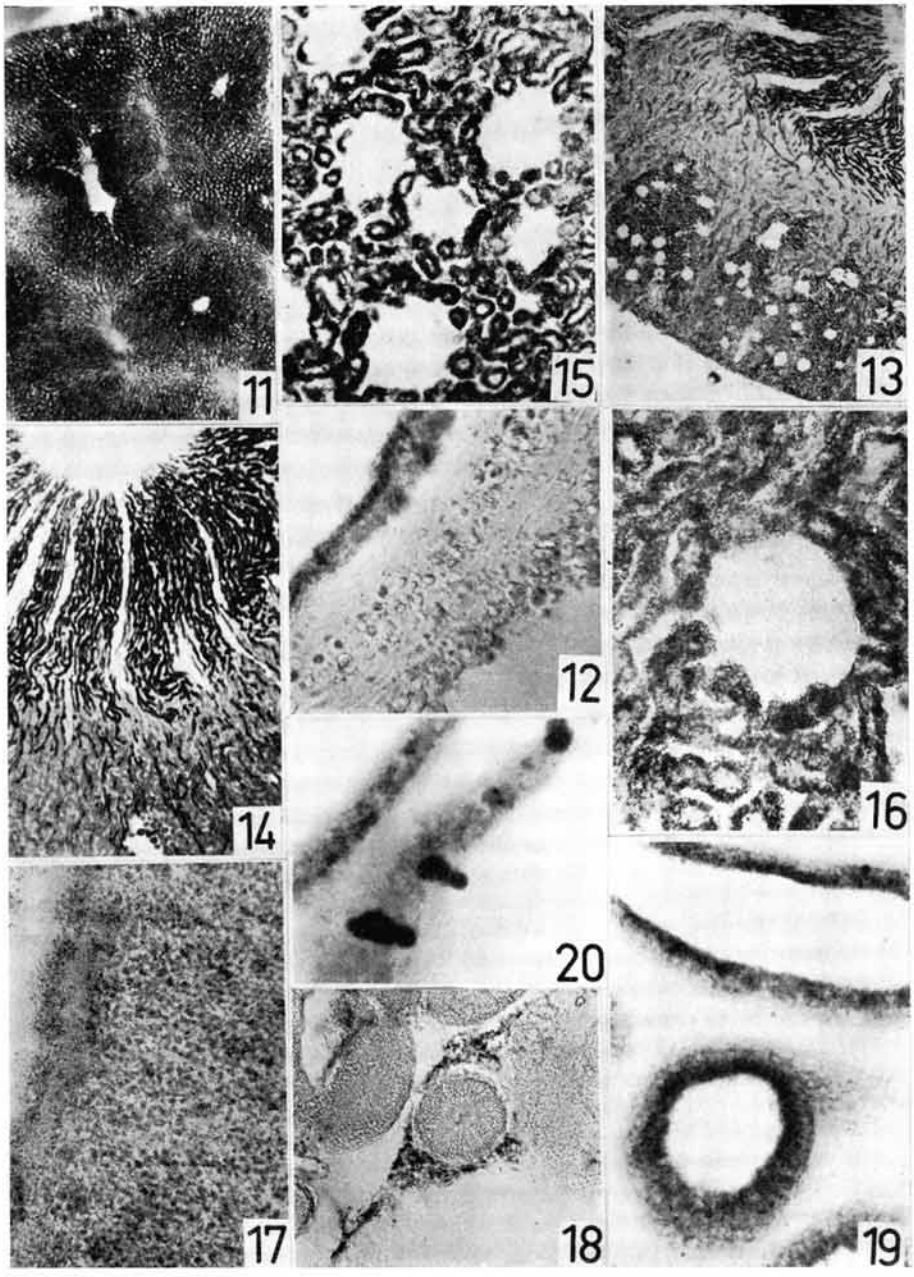

Fig. 11. Liver Very intense reaction in the periportal area of the hepatic lobule. – *Fig. 12.* Cross section of the trachea. Positive reaction in the epithelium, and negative in the hyaline cartilage. – *Fig. 13.* Topographic view of the distribution

4. The Lymphatic Organs

The Spleen. There is a discrete reaction in the macrophages of the halo that surrounds the lymph follicles. No reaction occurs in the lymphoid tissue, but a diffuse, finely granular reaction is present in the red pulp.

Lymph Nodes and Bone Marrow. The reaction is negative.

5. The Urinary System

The kidney is very rich in succinic dehydrogenase, the enzyme being distributed in it very systematically. It is the organ which has been studied chiefly both as regards the normal distribution and that resulting from the effect of mercurial diuretics [11, 12, 17, 18, 19, 20, 21]. According to its enzyme content, the kidney can be divided into three portions: The cortex, showing a very intense positive reaction, and the medulla, having a completely negative reaction. Between the two there is an "intermediate zone" presenting an outer, faintly positive layer, and an inner strongly positive layer. The boundary between the latter and the negative medulla is particularly sudden and devoid of any transition (fig. 13).

The "intermediate zone" corresponds in *Heidenhain*'s terminology to a special region of the kidney having specific functional and circulatory characteristics. It is in this region that experimental deposits of calcium occur in rats given Na2HPO4 [28], and it presents peculiarities in its circulation [29]. The distribution of succinic dehydrogenase is another criterion for making a topographical distinction of this "intermediate zone" as one having histochemical reactions of its own, different from those of the medulla (fig. 14).

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of the succinic dehydrogenase in the kidney. Central zone intensely positive, except for glomeruli. Negative medulla. "Intermediate zone" with its two layers—the inner showing an intense reaction, the other a weak reaction. – *Fig. 14.* Kidney. "Intermediate zone". Above is seen the totally negative medulla. – *Fig. 15.* Kidney. Cortex. Totally negative glomeruli, surrounded by intensely positive proximal convoluted tubules. – *Fig. 16.* Same as fig. 15, but with greater magnification. – *Fig. 17.* Adrenal cortex. Intense reaction in the glomerulosa, and rather less in the fasciculata. The sudanophile zone contains very little enzyme. – *Fig. 18.* Testis. Positive reaction in the interstitial cells. – *Fig. 19.* Tubules of the epididymis. Intense reaction in the epithelium showing grains located near the apical poles. – *Fig. 20.* Epithelium of epididymis. Coarse grumes with succinic dehydrogenase reaction, in connection with the secretion.

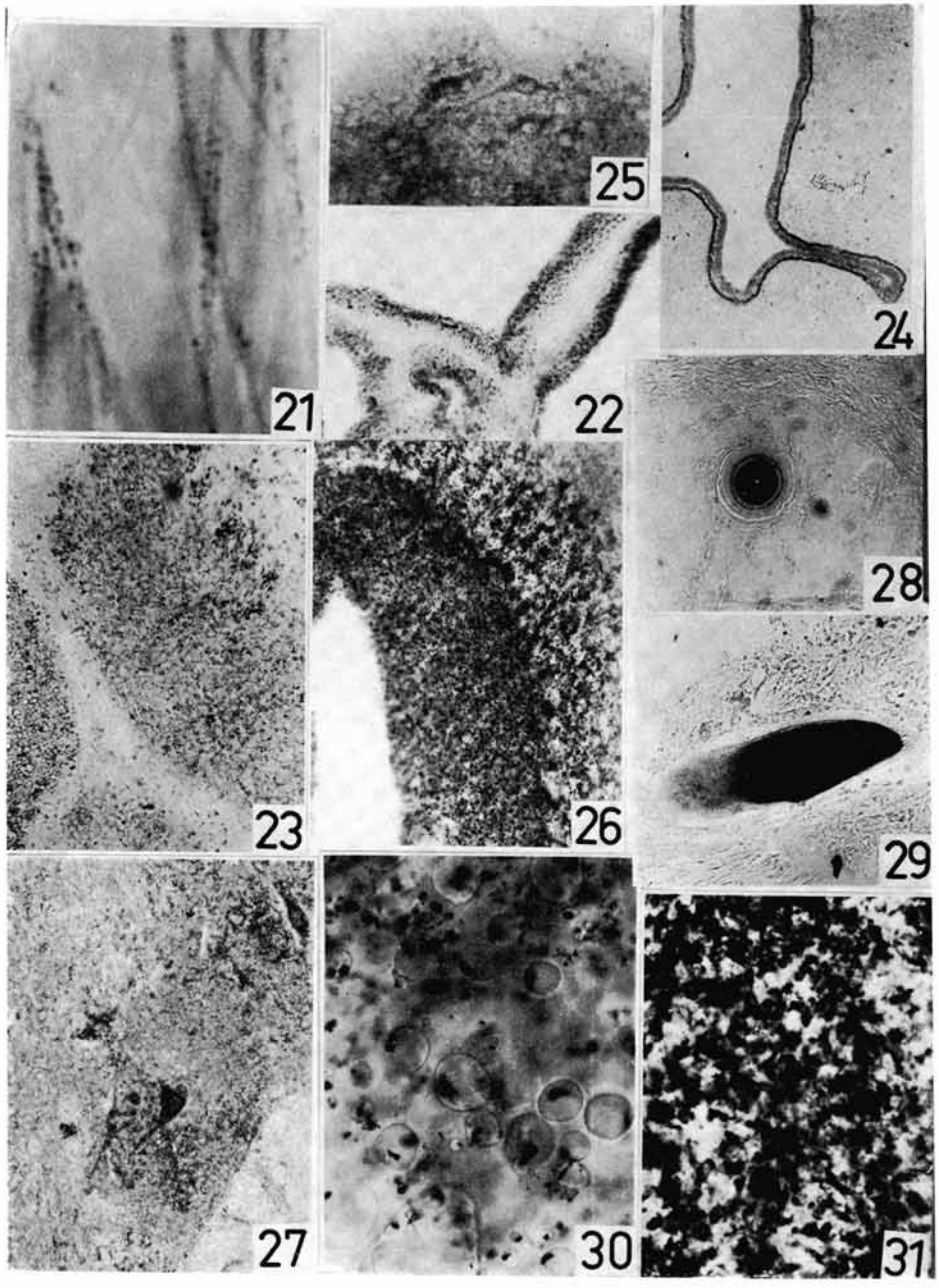

Fig. 21. Spermatozoa in the epididymis Granular reaction in the spiral thread. – *Fig. 22.* Intense reaction in the prostatic epithelium. – *Fig. 23.* Ovary. Positive reaction in the corpora lutea and interstitial gland. – *Fig. 24.* Uterine horn. Re-

Studying the reaction in the various parts of the nephron we find that in the glomeruli the reaction is negative; in the proximal convoluted tubuli very intense, granular or in the form of minute rods (figs. 15 and 16). Not infrequently needle-shaped crystals are found, due to much precipitation of formazane. The reaction very probably takes place in the chondriome, although it is not easy to determine its location; the nucleus is always negative. The straight, terminal portion of the proximal convoluted tubule shows a weaker reaction than the rest of the tubule, and this is probably the cause of the lightly stained part of the "intermediate zone". The descending limb of the loop of *Henle* and the collecting tubules are completely negative. The ascending limb of the loop of *Henle* is intensely positive, being probably the part of the nephron which contains most succinic dehydrogenase; nevertheless, this reaction is not uniform throughout the length of the ascending limb, being much more intense in some parts than in others. The second (distal) convoluted tubule is intensely positive, whereas the straight collecting tubules are completely negative.

Variations in distribution have been described according to the species of animal concerned [31], and changes due to the administration of mercurial diuretics [17, 18, 19, 20, 21] and of other substances [22].

In the bladder the reaction is mildly granular, both in the epithelium and in the smooth muscle.

6. The Endocrine System

The Adrenals. In general, the reaction is intense in the cortex and practically negative in the medulla where only a few scattered granules are to be seen. In the cortex, the reaction is intense in the zona glomerulosa, and in the zona fasciculata, and distinctly weaker in the zona reticularis. Between the glomerulosa and the fasciculata there is a continuous layer in which the reaction is weak, corresponding to the sudanophile layer described by *Deane* and *Greep* [30]

action near the basal poles in the epithelium. – *Fig. 25.* Cerebrum. Granular reaction in the cytoplasm of the soma, and in the proximal part of the dendrites in the neurones. – *Fig. 26.* Cerebellum. Intense reaction in the molecular and granular layers. Intense positive reaction in *Purkinje* cells. – *Fig. 27.* Motor neurones in the spinal cord. – *Fig. 28.* Cross section of hair bulb. – *Fig. 29.* Oblique section of hair bulb. – *Fig. 30.* Fat cells from the neighbourhood of the kidney. Needles of formazane and diffuse staining of fat droplet. – *Fig. 31.* Brown interscapular adipose tissue showing very intense reaction.

(fig. 17). The adrenal cortex shows a striking reaction in this particular layer when it is incubated without a specific culture medium, i.e., by endogenous reduction of the tetrazolium [12].

Within the cells the reaction appears in the shape of granules in the cytoplasm and as a diffuse staining of the lipid droplets, probably due to secondary dissolving of the formazane by the lipids.

The Pituitary. In the pars glandularis the reaction is negative. In the pars nervosa two types of reaction are seen: a granular reaction with small regularly distributed granules whose position in the cell we have not been able to determine, and scanty large drops in which the reaction appears as a red stain.

The Thyroid. We have constantly obtained negative reactions. Nevertheless, using endogenous substrates it has been possible to show the presence of a weak enzymatic action which disappears in frozen material [13].

7. The Male Reproductive Organs

The Testis. The reaction is clearly positive in the interstitial cells. There are a few granules in the seminiferous tubules, but their exact location has not been determined (fig. 18). The spermatozoa within the seminiferous tubules show a similar reaction to that described below in connection with the epididymis.

The Epididymis. The epithelium shows an intense granular reaction, the granules being most numerous near the apices of the cells (fig. 19). Red grumes of varying size are also seen, sometimes in relation to centres of succinic dehydrogenase activity (fig. 20). They are probably masses of secreted substances. The spermatozoa within the lumen of the ducts of the epididymis show a marked granular reaction, clearly limited to the spiral thread of the middle piece (fig. 21).

The Prostate. In the epithelial cells of the ventral prostate there is a marked granular reaction, situated at both poles of the cells (fig. 22). The interstitial tissue is negative. The lobes of the dorsolateral prostate are also positive, but less strongly so.

Seminal Vesicles. The epithelium presents an intense positive reaction.

8. The Female Reproductive Organs

Ovary. There is a frankly positive reaction in the interstitial gland, and rather less so in the corpus luteum (fig. 23). In the ovary

of the rabbit very striking experimental changes have been seen in the distribution and intensity of the reaction after the injection of chorionic gonadotropin [6, 7].

The Uterus. Endometrium: The superficial epithelium and the glands in the horns of the fundus show a strongly positive reaction. The reaction is granular, the majority of granules being situated near the basal pole of the cell (fig. 24). In the myometrium there is a weak, diffuse, granular reaction. In the body of the uterus the reaction is decidedly weaker.

Vagina. The epithelium presents a positive reaction varying with the phases of the ovarian cycle.

9. The Nervous System

In general the reaction is clearly positive in the grey matter and very weak or even negative in the white matter. The cytoplasm of the nerve cells presents a granular positive reaction which spreads into the proximal part of the dendrites (fig. 25).

The Brain. The cortex shows an intense granular reaction, particularly evident in the second and fourth layers.

The Cerebellum. The reaction is intense, granular, but patchy and not homogeneous in the inner granular (nuclear) layer. In the outer molecular layer there is a marked granular reaction, but not so strong as in the inner granular layer. The *Purkinje* cells show an evident granular reaction (fig. 26).

The Spinal Cord. In the motor cells of the anterior horns, there is an intense granular reaction (fig. 27). A diffuse granular reaction occurs in the grey matter, but none in the white matter. In the ependymal cells there is a discret granular reaction.

10. Sundry Tissues

Skin. We have used rat tail skin which is very suitable for study. The epidermis shows a weak granular reaction. The deep layers are frankly positive, whilst the horny layers are negative. The sebaceous glands are weakly positive, as also are the hair root sheaths. The hair bulbs of some follicles present an intense reaction (figs. 28 and 29). This reaction is the most intense that we have observed in any tissue whatever, and has the further peculiarity of being homogeneous. Similar reactions have also been described in the skin of human beings and of guinea-pigs [9, 10].

Adipose Tissue. We have studied this tissue in the superficial

fascia, the mediastinum, the neck and the perineal region. In general it shows an evident reaction which may at times be intense. The reaction appears as a bundle of needles, or as a grume in the cytoplasm near the nucleus of the fat cell (fig. 30). The fat contained in the cells usually stains red, probably through the colouring matter dissolving in the fat.

The brown adipose tissue presents a very intense reaction. In the fat cells can be seen bundles of needles or crystals of colouring matter situated in the cytoplasm (fig. 31).

Bone. We have studied the ribs and tibia. The findings were negative.

Summary

The histochemical technique which acts by reducing tetrazolium salts, demonstrates the distribution of succinic dehydrogenase in numerous normal tissues and organs of the albino rat. The liver, kidney, striated muscle and brown adipose tissue show the most intense reaction. This is also the case with the gastric and intestinal mucosae, and with the epithelium of the male and female reproductive organs.

The reactions may be confined very selectively to certain portions of an organ, such as the periportal area of the hepatic lobule, or the cortical zone of the kidney, or to particular types of cells; in the striated skeletal muscles, only the slender "red" fibres show an intense positive reaction: in the thick "white" ones, the reaction is much weaker or even negative. Some types of cells present a remarkably intense reaction, as can be seen in the parietal cells of the gastric mucosal glands. It is significant that we found the most intense reaction in the hair bulb.

Résumé

La technique histochimique qui comprend la réduction des sels de tétrazolium démontre la distribution de la succinodéhydrogénase dans les tissus. Les auteurs ont étudié de cette façon un bon nombre de tissus chez le rat blanc. Le foie, les reins, les muscles striés et le tissu adipeux donnent la réaction la plus intense. C'est également le cas pour les muqueuses gastro-intestinales et l'épithélium germinatif des organes génitaux des deux sexes.

La réaction peut être sélectivement localisée à certaines portions d'un organe, par exemple à la région périportale des lobules hépatiques ou à l'écorce du rein; elle peut aussi se localiser à certains types cellulaires: dans les fibres musculaires striées, seules les fibres minces «rouges» donnent une réaction très positive, alors que les fibres «blanches», plus épaisses, sont négatives ou se colorent moins intensément. Dans certains types cellulaires la réaction est particulièrement intense, ainsi dans les cellules bordantes de la muqueuse gastrique. Il est intéressant de relever que le bulbe pileux présente la réaction la plus forte que les auteurs aient observée.

Zusammenfassung

Durch Reduktion von Tetrazoliumsalzen kann das Vorhandensein von Succinodehydrogenase in zahlreichen normalen Geweben und Organen der weißen

Ratte nachgewiesen werden. Die stärkste Reaktion zeigt sich in der Leber, den Nieren, der quergestreiften Muskulatur und dem braunen Fettgewebe. Eine starke Reaktion findet sich auch in der Magen-Darm-Schleimhaut und im Epithel der männlichen und weiblichen Fortpflanzungsorgane.

Es kommt vor, daß die Reaktion nur auf ganz bestimmte Abschnitte eines Organs beschränkt ist, wie z. B. auf das Gebiet der Pfortaderäste im Leberläppchen oder auf die Nierenrinde oder auf ganz besondere Zelltypen. Im quergestreiften Skeletmuskel zeigen nur die dünnen roten Fasern eine stark positive Reaktion, während die dicken weißen Fasern wesentlich schwächer oder sogar negativ reagieren. Manche Zelltypen weisen eine besonders starke Reaktion auf, wie z. B. die Wandzellen der Magen-Schleimdrüsen. Bemerkenswert ist, daß die Haarbulbi die weitaus stärkste Reaktion zeigen.

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