

THE HEART IN THE EXPERIMENTAL HYPER- THYROIDISM WITH SPECIAL REFERENCE TO ITS HISTOLOGY

HIROTOSHI HASHIMOTO

(From the Laboratory of Prof. K. Miura's Medical Clinic,
Imperial University, Tokio, Japan.)

INTRODUCTION

It is generally known that cardiac disturbances occur in patients suffering from goitre, in not only the exophthalmic type, but also apparently simple goitre, and that cardiac weakness is one of the most frequent causes of sudden death. The subject is, therefore, one of great interest to clinical and experimental investigators.

These cardiac disturbances may occasionally be caused by pressure of the tumor upon neighboring structures—venous or respiratory obstruction, or mechanical affection of the sympathetic or vagus nerves (“mechanical goitre heart”—A. Kocher). In the majority of the cases, however, they may be attributed to a certain kind of intoxication, concerning the etiology of which there is a diversity of opinion. Bircher has claimed that the cardiac disturbances associated with endemic goitre are due, not to the goitre itself, but to the general intoxication which is responsible for the changes in the thyroid glands. Most observers believe, however, that the cardiac manifestations associated with goitre arise for the most part from thyroid intoxication (especially in Graves’ disease) and chiefly from an excessive stimulation of the cardiac accelerator nerves.

Concerning the histology of goitre hearts, fatty degeneration of muscle-fibers appeared to be the only lesion ever found by the observers previous to Fahr (1916), who recently found evidence of chronic interstitial inflammation occurring in the heart muscle. In his case of Graves’ disease, as well as of simple goitre, there were collections of round cells, chiefly lymphocytes, among the muscle-fibers and in the neighborhood of the blood vessels. In the large myocarditic areas the muscle-fibers were separated from one another and showed various degenerative changes, i. e., fragmentation, dissolution, etc. Scattered among

the lymphocytes in these areas were a number of fibroblasts, while in the smaller areas there were lymphocytes only. Fatty degeneration of muscle-fibers was observable only to a limited extent.

Upon the basis of the evidence above cited, Fahr considered that the toxin circulating in the body of a goitre patient may cause not only an excessive stimulation of the cardiac nerves, but further have a direct effect upon the heart muscle itself.

Recently I had an opportunity to examine the hearts of two exophthalmic goitre patients, and was able to confirm the findings of Fahr, lymphocytic infiltration between the muscle-fibers or around the blood vessels being observed.

For solving the question of the etiology of the goitre heart, it is of the utmost importance to investigate the cardiac manifestations in artificial hyperthyroidism, usually produced experimentally by thyroid administration, and to compare them with those in human subjects suffering from goitre.

Tachycardia was produced in various animals by means of thyroid administration by Ballet and Enriquez, Canter, Georgiewski, Hellin, Lanz, Angiolella, Ludke, Lepine, Peiser, Edmunds, Pfeiffer, v. Fürth and Schwartz, while Paessler and Schulze, Marine and Lenhart, Cunningham, and Carlson and his pupils failed to demonstrate its occurrence in thyroid-treated animals. Farrant (1913), by his careful investigations (recording accurately the pulse rate of thyroid-fed cats by the aid of a polygraph), later confirmed the fact that thyroid feeding induces a steady increase in the heart beat, which subsides gradually after the cessation of the feeding.

With regard to the anatomical changes in the heart resulting from thyroid administration there are a number of observations. Iscovesco (1913) found that repeated injections of thyroid extract (an ether-soluble substance, isolated from thyroid tissue) produced in rabbits hypertrophy of the heart associated with enlargement of many other internal organs (suprarenals, ovaries, uterus, spleen, and kidneys). E. R. Hoskins (1916) fed small amounts of thyroid tissue to young albino rats for a long while and observed a decided hypertrophy of heart, liver, spleen, kidneys and suprarenals. Herring (1917) obtained results which were in general agreement with those of Hoskins, differing only in the degree of the hypertrophy, which in the heart, suprarenals and kidneys was larger by far. Hoskins

ascribed the hypertrophy of the organs to the increased rate of metabolism promoted by thyroid feeding, while Herring regarded an increased production of adrenalin in the body as an additional factor. The results of the recent research by Hewitt (1920) tend to show that the hypertrophy of the internal organs produced by thyroid administration is temporary, and that a return to normal proportions is indicated after the cessation of thyroid feeding.

Concerning the histology of the heart in artificial hyperthyroidism, only a few observations are recorded. Farrant (1913) found in the hearts of thyroid-fed cats and rabbits morphological changes varying from a condition of general wasting of muscle-fibers to what may be described as a condition of hyaline degeneration. Finding that the muscle-fibers were swollen, with few nuclei and no transverse striations, and that both the fibers and the nuclei stained poorly, he termed these changes "myocarditis without evidence of inflammation." Herring (1917) stated that the enlarged hearts of thyroid-fed rats showed upon microscopic examination an increase of muscle-fibers and many of them an apparent increase in size.

In view of the results of the investigations briefly cited above, it would appear that the cardiac changes resulting from thyroid administration are similar to those known as the changes in "goitre heart" in human subjects, but by no means form the complete picture of the latter, as is shown in the histology of the heart—in experimental hyperthyroidism and in goitre. According to the foregoing investigators, it is at most a hyaline degeneration that has been produced experimentally in the heart muscle of animals by thyroid administration, whereas in human goitre heart even marked myocarditic lesions have been observed (Fahr). As long as we fail to identify the cardiac disturbances resulting from an experimental hyperthyroidism with those of human goitre hearts, we are not justified in assuming that the cardiac disturbances associated with goitre arise from thyroid intoxication or from an excess of thyroid secretion in the body.

I have long been aware that, in the course of the experimental hyperthyroidism produced by thyroid feeding in albino rats, sudden death is by no means infrequent, and that there are occasionally findings which enable us to ascribe such death

to a cardiac insufficiency. Because of a suspicion that more severe changes than a hyaline degeneration might be detected microscopically in these hearts, I undertook to investigate the histology of the hearts of a large number of albino rats, either killed or dying during the course of thyroid administration.

EXPERIMENTAL DATA

(1) The effect of feeding with toxic doses of thyroid upon the structure of the heart.

In this series of investigations the material examined was obtained from 38 thyroid-fed albino rats, 28 of which were killed and 10 found dead during the course of the experiment. The experiment was begun with 53 animals chosen for thyroid administration. Of these, 25 died, but 15 had been dead so long when discovered that the visceral organs were unfit for histological use. Each animal was kept in a separate wire-net cage, in a large cement-floored animal room, light, dry, warm, well-ventilated, and clean. The food was bread and milk mixed fresh each day, and green vegetables on alternate days. The thyroid administered was exclusively desiccated powder which had been prepared by the writer himself from fresh thyroid glands by the aid of Faust's apparatus, at a low temperature of 35-40° C. Various amounts of this desiccated thyroid, containing 0.488 per cent. of iodine, were added to the food daily—to 14 males, 0.5 grms.; to 9 males and 9 females, 0.3 grms; to 10 males, 0.2 grms.; to 11 males, 0.1 gm. As controls a number of healthy rats were kept in cages in the neighborhood of the experimental animals, and were fed on similar food, with the addition of corresponding amounts of desiccated beef in place of the thyroid.

Under the influence of 0.1-0.5 grms. of desiccated thyroid per day most of the animals lost appetite for food after three or four days and became inactive and weak, lying flat on the floor. Some of them, being exceedingly susceptible to the toxic effect of the thyroid, died during this period. Most of the animals, however, survived the period of depression and became apparently vigorous in the second week, some of them appearing to be rather nervous and excited. A large number died about this time, the moribund condition (dyspnoea, hypersalivation, etc.) setting in very suddenly. A few survived longer than two weeks. Such animals were liable to become abnormally irritable and voracious. Despite an increased consumption of food, however, they became emaciated and died eventually, sometimes very suddenly. In one case a sudden death was evidently evoked by over-exertion. This was a male rat that had been receiving 0.5 grms. desiccated thyroid daily for three weeks, but which had been apparently in a vigorous condition; it died very suddenly after being chased by another excited male rat. On dissection there was no trace of wound or bleeding to which the sudden death might be attributed. The cause was probably cardiac insufficiency.

As to changes in the body weights of the thyroid-fed animals, an actual diminution was observable in only a few, whereas the gain in weight was noticeably less in the thyroid-fed animals than in the controls, kept for the same length of time under similar dietetic and environmental conditions; the differences varied from 16 to 34 per cent. per ten days. The rats receiving buccal administration of the above doses of thyroid, if not killed before that time, all died within three weeks. The average duration of life under the influence of such thyroid administration was eleven days.

At various intervals during the course of the thyroid feeding twenty-eight animals were killed by chloroform and completely bled. The hearts were cut off from the blood-vascular trunks, cleansed of blood and the auricles and ventricles slit up. The other internal organs were also dissected out. After being weighed these were preserved in 10 per cent. formol solution. For microscopic examination sections 5 μ thick were made from the heart muscle and other organ tissues and stained with haematoxylin—eosin, haematoxylin—Sudan III, in some cases Giemsa's stain (used according to Schridde) or methylgreen—pyronin stain (Pappenheim-Unna).

The heart weights of the thyroid-fed animals were compared with those of the controls in terms of weight per 100 mm. body length, i. e., distance from the tip of the nose to the center of the anus. The measurement was made immediately after death, before rigor mortis set in. The average weight of the hearts per 100 mm. body length in the several series of thyroid-fed males varied from 0.536 to 0.677 grms., that of the controls averaged 0.510 grms. Percentage differences are, therefore, from 5 to 33 per cent. In the females the average weights of the hearts per 100 mm. body length were 0.549 grms. and 0.408 grms. in thyroid-fed and control animals respectively, the percentage difference being 34 per cent. If the heart weights of my control rats be compared with those given by Donaldson for the Wistar rats of equal body length, the hearts of my normal males average 32 per cent. and those of my females 6 per cent. heavier than those given by Donaldson. According to the latter's method, the increase in weight of the hearts ranges from 17-77 per cent. in thyroid-fed males, and 43 per cent. in thyroid-fed females.

Microscopically the hearts of thyroid-fed rats that were killed differed little from those of the control normal animals except in size, which had increased more or less markedly in the thyroid-fed animals. The hearts of the experimental animals that were found dead were conspicuously dilated, especially in the right ventricle, and a marked degree of passive congestion was noted. The coronary vessels and their branches were distended with blood. Much fluid blood with little clot was present in the cavities. Dropsy in the pericardial cavity was, however, rarely found. On macroscopic examination the myocardium and valvular apparatus showed no apparent change. Rigor mortis of the heart muscle obviously set in earlier in the thyroid-fed animals than in the normal controls. This was also true of the muscular tissue in general, and an acidosis in the experimental hyperthyroidism, noted first by Kuriyama, might bear a certain relation to the early occurrence of rigor mortis.

Upon histological examination of the hearts of these thyroid-fed rats the muscle-fibers were found in most cases to be swollen and separated. The cross-striations appeared less clear than normal. Vacuolization occurred rarely, but there was no fatty degeneration, which fact was determined by staining with Sudan III. Many nuclei were larger than normal, and the occurrence of double nuclei more frequent than normal. On the other hand, a number of nuclei showed an accumulation of chromatin particles along the axis, impaired staining, pycnosis, etc. Not only the muscle fibres but also the cells of the interstitial connective tissue were seen to be swollen, and appeared also to be increased in number.

Besides such vague and diffuse changes, the heart muscle of the thyroid-fed rats showed other very obvious changes within certain circumscribed scattered areas. Under the low power they appeared as dense patches consisting chiefly of round cells. These formations were elongated, spindle-shaped or pyramidal, as the cells were collected in the narrow spaces between the muscle-fibers. They were

found to be situated frequently in the neighborhood of the blood vessels. Under the high power it was seen that the accumulation consisted chiefly of large peculiar cells, most of which were about three or four times the size of a red blood cell. Their protoplasm was slightly basophilic and outlined faintly and irregularly. The nuclei were situated eccentrically, were quite large in size, round, oval, indented, occasionally bent or twisted in shape, and pale in color, showing few chromatin particles. In their morphology, therefore, these cells simulate the large mononuclear or transitional cells present in circulating blood. They were accompanied by some smaller cells with dark nuclei containing a dense, thick chromatin network, and correspond both in size and appearance to lymphocytes present in the circulating blood. Among these cells, especially in comparatively large areas of changes, a number of fibroblasts were found scattered or accumulated. These had large, pale, oval or elongated nuclei with little chromatin and one or two distinct nucleoli; the similarly elongated protoplasm was faintly outlined and furnished with irregular processes. Some of the fibroblasts were swollen and in appearance resembled the large cells described above as the main constituents of the cell-collections. Some of the elongated cells were conspicuous for the chromatin structure, the chromatin particles accumulating along the axis of an oval or elongated and sharply outlined nucleus. These cells are no more or less than the "Myocyten" of Anitschkow. Not infrequently there were cells showing mitotic figures. A few polymorphonuclear leucocytes were occasionally found. The muscle fibres adjacent to the cell-formations above described were pushed aside by these. In the neighborhood of the smaller areas of myocarditic changes they appeared usually to remain practically intact, whereas the fibers present around or taken within the larger areas were severely disturbed, being vacuolated or dissolved. The nuclei of such degenerated muscle-fibers showed various stages of disintegration, such as pycnosis, karyolysis, etc. In places, however, some of the nuclei were seen to be less severely injured, and thin rings of muscle substance were left surrounding them.

The histological appearance of the cell collections varied considerably, owing to the number and proportions of the number of the cellular elements. In some small areas of myocardial changes there were mainly lymphoid cells, whereas in some of larger areas, usually at a later period of thyroid feeding, fibroblasts, and occasionally myocytes, prevailed over other cell types. It is interesting to note that at later periods of thyroid feeding the histological structure of the myocardial changes tended to vary considerably in the different areas, the cells of large mononuclear type or of lymphoid type accumulating in some areas and fibroblasts in others.

As to the seat of the myocardial changes it is common knowledge that these are most likely to occur in the neighborhood of the blood vessels, or beneath the pericardium. Occasionally, however, in my experiments, especially in later stages of thyroid feeding, they were to be found in any part of the myocardium. The posterior wall of the left ventricle near the base was frequently affected, though not always. The present investigation did not enable me to decide definitely whether or not the bundle of His may be involved in the lesion.

The severity of the myocarditic lesions, judged by the size and number of their areas, varied considerably according to the factors involved. They tended to become more marked according to the length of time covered by the thyroid administration. Although small cell-collections were observable in the heart muscle of animals killed as early as three or four days after thyroid administration had begun, the areas of marked myocarditic lesion, large in size and number, were

usually found in animals that had been killed or found dead about the end of the second week or later. The size of the thyroid dosage also may determine the severity of the myocarditic lesion. The animals fed with larger doses exhibited more marked changes and in a larger number of cases than those fed with smaller doses for similar lengths of time and under similar environmental and dietetic conditions. Myocarditic lesions were found in 90 per cent. of the rats killed in the course of administration of 0.5 grm. desiccated thyroid per day, while they occurred in only 50 per cent. of the animals killed in the course of administration of 0.3 grm. per day. The third and most important factor is the individual susceptibility of animals to the effects of thyroid administration. Owing to this factor, an animal fed with a smaller dose for a shorter period may exhibit a more marked lesion of the myocardium than one fed with a larger dose over a longer period. The more severe lesions were noted more frequently in the animals that were found dead than in those that were killed. Such a difference might be attributed chiefly to the fact that the material for the microscopic examination was obtained from the animals that were killed at comparatively early stages of thyroid administration—from the second to the fifteenth day, and from those that had died at comparatively later stages of thyroid feeding—from the twelfth to the twentieth day.

(2) *The Effect of Feeding With a Non-toxic Dosage of Thyroid Upon the Structure of the Heart.*

In this series of experiments eleven adult albino rats, six males and five non-pregnant females, were given non-toxic doses of desiccated thyroid, 0.03 grm. on alternate days. All the animals but one remained in an apparently healthy condition for a long period under the feeding, and were killed at intervals ranging from 37 to 170 days. One of the males died after 109 days, but the organs were not examined microscopically. The details of technic are as already described, and need not be repeated.

The hearts of these thyroid-fed animals, as compared with those of the normal control animals, were markedly enlarged in size and weight. The heart weight of the males averaged 0.600 grm., and that of the females 0.496 grm., an estimated increase of 18 to 22 per cent. respectively, based upon the average weights of the controls, which were 0.510 grm. for the males and 0.408 grm. for the females. If the average weights of the hearts be compared according to Donaldson's method, the increase resulting from thyroid feeding is 26 per cent. in the males and 27 per cent. in the females.

The gross appearance of the hearts of these thyroid-fed rats was quite similar to that of the controls, except for the increase in size and weight. On microscopic examination, however, one of five males and three of five females exhibited small areas of myocarditic changes. Compared with the lesions observed in the animals receiving toxic doses of thyroid, those seen in the animals in this series were conspicuous for the smaller size of the affected areas and the greater variation in the proportion of the constituent elements in different areas. Some of these areas were composed chiefly of round wandering cells, while in others fibroblasts prevailed. In one of the males showing no inflammatory change in the myocardium there was a slight proliferation of the connective tissue without any inflammatory reaction in the neighborhood of the blood vessels.

(3) *The Histology of Normal Hearts. The Histology of the Hearts of Animals Dying from Other Causes Than Thyroid Intoxication.*

In order to decide whether or not the occurrence of the myocarditic lesions above described was due to the toxic effect exerted by

the thyroid substance itself, I made a series of control investigations.

The hearts of 22 normal and healthy adult albino rats were examined post-mortem; five of these animals were non-pregnant females and the remainder males. Most of them had been receiving desiccated beef in varying amounts.

The microscopic appearance of the heart muscle of the normal albino rats agreed in general with that of rabbits, a detailed description of which has been given by Anitschkow and also by Kiyono. The myocarditic lesions described above were not observed in a single instance.

Since the most marked myocarditic lesion had been found in the animals dying during the course of thyroid feeding, it was thought desirable to investigate the condition of the heart in the animals dying from other causes than thyroid intoxication. One of these rats, in which death was due to diarrhea, showed no myocarditic changes.

A histological examination was next made of the heart muscle from nine male adult rats in which death followed thyro-parathyroidectomy, one immediately after the operation, four within 18 hours, and the other four after two or three days. The hearts of these animals exhibited neither cell infiltration nor fibrosis, although congestion and edema of the myocardium was quite marked in five of them.

The research was next directed to an investigation into the histology of the hearts of rats dying after repeated overexertion at a high temperature, for there is a certain similarity between the condition of severe thyroid intoxication and that of overexertion at high temperature, especially when there is great excitation of heart action. Zwaskewitsh found that the hearts of animals exposed to high temperatures for a considerable period of time showed a cloudy swelling of the muscle-fibers. Litten, Naunyn, Nasaroff, Werhovsky, and Welch found fatty degeneration of the heart muscle instead of a cloudy swelling.

As to the histology of the hypertrophied hearts, in which hypertrophy is the result of experimental valvular lesions, there are a number of studies on record. Stadler and many others found a diffuse increase of connective tissue but no inflammatory changes in the heart muscle.

Through the courtesy of Doctor S. Sugahara, I had an opportunity to examine the hearts of five albino rats (four males and one female) which had died after having been exposed to temperatures of from 30° to 40° C, and at the same time forced to overexertion for two or three hours in a warm revolving box, the procedure being repeated several times at intervals of a few days.

On histological examination myocarditic lesions such as were shown after thyroid administration were not found in any of these animals. A pregnant female rat showed a slight proliferation of the interstitial connective tissue around the blood vessels, but this was not accompanied by infiltration of any of the wandering cells.

(4) Thyroid Administration Combined With Vital Staining With Carmine.

In order to establish the nature of the various cells constituting the myocarditic lesions above described, I carried out another series of experiments, employing the method of vital staining with carmine, as Kiyono did in his investigations of the cellular elements of granulation tissue produced experimentally in the hearts of rabbits.

Six young adult male albino rats, weighing from 75 to 85 grms., which had been receiving 0.1 gm. desiccated thyroid daily in food, were injected intravenously with 0.05-0.15 cc. of a 4 per cent. solu-

tion of carmine in saturated lithium carbonate on five successive days. The animals exhibited various symptoms referable to the thyroid intoxication (anoroxia, hyperexcitability, emaciation, etc.) and were killed after having received five or six doses of thyroid and 12 to 18 hours after the last injection of carmine. The efficiency of the aforementioned dose of thyroid had been estimated by a preliminary experiment in which six rats of the same sex and similar size, receiving this dosage daily, had died after six or seven days.

In a second experiment four male rats, weighing 140 to 150 grms., and having received non-toxic doses of desiccated thyroid (0.003-0.03 grms. on alternate days) for three months, were injected with 0.1-0.2 cc. of the carmine solution per day, for five successive days and then killed. Thyroid feeding was commenced when the animals weighed only 40 to 50 grms., and the dosage was increased gradually as the body weight increased. The animals thrived and growth was neither retarded nor accelerated, as compared with the control animals. The hearts and other organs, however, were found to be somewhat enlarged.

In a third series, six healthy male rats, fed on milk and bread with or without the addition of desiccated beef, received the injection of carmine by a similar technic.

The histology of the hearts of these normal albino rats, examined after vital staining with carmine, was in general much the same as that of normal rabbits, described in detail by Kiyono, except as concerned the myocytes of Anitschkow. In the interstitial connective tissue of the heart muscle are a number of cells containing a greater or lesser number of cytoplasmic granules which stain with carmine. It has been proved by Kiyono that these cells correspond to the "clasmocytes" of Ranvier. Besides the clasmocytes, the myocytes of Anitschkow are found scattered in the interstitial connective tissues of the myocardium. According to Kiyono these cells possess no cytoplasmic granules which stain vitally with carmine. So far as I am aware, however, it is evident that the majority of the myocytes present in normal as well as in pathological heart-muscle contains these granules, varying in size from fine dust-like particles to globular bodies large enough to be easily seen by a moderately high power microscope. There is an apparent tendency for the granules to be small in size and few in number in cells with more basophilic cytoplasm, while on the other hand they appear to be large and more numerous in cells with clear, swollen cytoplasm. The fibroblasts and the endothelial cells of the capillaries contain no vitally staining granules. The cells of the muscle-fibers likewise do not stain vitally with carmine nor do they exhibit any carmine granules.

By the aid of a vital staining with carmine the nature of the cell elements in the regions of myocarditic changes, resulting from thyroid administration, was made more obvious. In the rats receiving toxic doses of thyroid there appeared an increase in the number of the vitally staining cells—"Histiocytäre Zellen" of Kiyono—throughout the interstitial tissue of myocardium. A large number of these cells were swollen and had relatively shorter processes than normal cells, and some had even rounded up. The cytoplasm was densely packed with carmine granules and often vacuolated. Not infrequently one or two of the carmine granules present in the cytoplasm were of enormous size, sometimes half the size of the nucleus. The nucleus was usually situated eccentrically and exceedingly irregular in shape, especially in the swollen polyhedral or round cells, where it was round, oval, polyhedral, indented, or even bent. The network of chromatin was for the most part quite thin, and its nodal thickenings stood out distinctly as chromatin clumps scattered in the clear karyoplasm, whereas the nuclei of the "histiocytäre" cells present in the normal

myocardium tend to show a thick and dense chromatin network. Mitotic figures are shown by some of them. The evidence of karyokinesis argues the rapid proliferation of these "histiocytaire" cells. These cells show a tendency to cluster between the muscle-fibers, or frequently in the neighborhood of the blood vessels, and to form patches of cell-collections. Judging from their morphological characters, we may easily identify the "histiocytaire cells," derived from clasmocytes, with the large peculiar cells which have been previously described as the chief constituents of the cell-collections found scattered in the myocardium of thyroid-fed rats. Among these cells is a fair number of myocytes conspicuous for the chromatin structure of their nuclei, some of which are much swollen.

The cell patches also contain other cells in varying numbers which do not stain vitally with carmine. Some of these are entirely indistinguishable from the lymphocytes present in the circulating blood, while other are quite similar in their morphological characteristics to the "histiocytaire" wandering cells, except that they contain no carmine granules. It is very difficult to decide whether the cells of the latter type are derived from lymphocytes, or whether they are "histiocytaire" cells which fail to exhibit carmine granules, owing to pathological changes in their cytoplasm. In certain cases such cells lacking carmine granules constituted the greater part of the cell-collections, together with a smaller number of clasmocytes containing fair deposits of carmine granules. Some of these cells are probably derived from the connective tissue, for they show one or two distinct nucleoli in a pale oval nucleus.

As mentioned above, the muscle-fibers in the normal hearts do not stain vitally with carmine, neither diffusely nor by granular formations, especially when the animals are killed later than half a day after the injection of the dye. In the hearts showing severe myocarditic changes, however, some of the muscle-fibers may stain diffusely pink with carmine. In such areas the cross-striations usually fade out. The nuclei therein may show various stages of disintegration, but this is not always the case. Though such a tinctorial change of muscle-fibers is seen in the hearts showing severe interstitial inflammatory changes, the two conditions appear to occur quite independently of each other. The muscle-fibers adjoining the interstitial cell-collections are not always stained pink. Red staining of heart muscle-fibers of animals injected *intra vitam* with carmine has been observed by Pari, by Masuda and by Kiyono, and interpreted by them as evidence of disintegration of the muscle-fibers.

Of four rats receiving non-toxic doses of thyroid over long periods, only one showed the myocarditic lesion described above. It is of interest to note that in the myocardium of this animal there were some myocarditic areas composed mainly of fibroblasts. Within such areas the "histiocytaire" cells containing carmine granules were not swollen, but flattened and elongated, with dark nuclei and a thick network of chromatin. The carmine granules were small and few in number. These observations suggest that these "histiocytaire" cells were no longer in an irritated, but in a stable condition, taking part in the formation of a fixed tissue. Nowhere were muscle-fibers stained pink.

(5) *The Effect of Inorganic Iodine Upon the Heart Muscle*

In order to decide whether the myocarditic lesions produced as the result of thyroid feeding were due to the effect of an abnormal increase in the thyroid secretion, or were simply due to the toxic action of iodine contained in the thyroid substance given, I administered an inorganic iodine salt (sodium iodid) to three albino rats that had survived a thyroidectomy.

A thyroidectomized female rat, which died after having received a buccal administration of 1 mg. sodium iodid per day for a week, showed no myocarditic lesions.

A male rat, weighing 78 grms., was injected subcutaneously with 10 mg. sodium iodid in a water solution ten days after a thyroidectomy and was killed four days later. There had occurred a fair degree of diuresis following the injection of the iodide. On microscopic examination the heart muscle showed a condition of edema, the muscle fibers being separated and the interstitial cells swollen.

Another male, weighing 69 grms., received a subcutaneous injection of 3 mg. sodium iodid in a water solution 10 days after thyroidectomy. In this case diuresis did not occur, but the amount of urine excreted tended rather to diminish. The animal became inactive and weak, and died two days after the injection. Gross and microscopic examination of the internal organs gave evidence of acute pneumonia, acute nephritis, a stasis of general blood circulation, and degeneration of the liver cells. The heart muscle showed marked myocarditic lesions in the wall of the left ventricle near the base. Fresh granulation tissue had formed beneath the pericardium, showing a tendency to grow toward the center of the wall along the interstitial tissue. In the area adjoining the pericardium the granulation tissue consisted chiefly of fibroblasts, whereas between the muscle-fibers it was composed mainly of large pale cells of mononuclear type and a few cells of lymphoid type. Muscle-fibers adjacent to the granulation tissue were vacuolated or dissolved, and the nuclei were scattered out showing various stages of disintegration. Histologically the myocarditic lesions found herein resembled so closely those resulting from thyroid feeding that one could scarcely distinguish the one from the other. The number of observations is too small, however, to warrant the drawing of final conclusions as to whether or not the myocarditic lesions, occurring as the result of thyroid administration, may be produced by the action of the iodine, which is not in the chemical combination proper to thyroid secretion.

A young female rat, not thyroidectomized, died after having received a buccal administration of 1 mg. potassium iodid 4 times a week. The myocardium showed no myocarditic lesion.

(6) *The Effect of Adrenalin Injection Upon the Histology of Heart Muscle*

K. Ziegler, in his experiments regarding the effect of repeated injections of small doses of adrenalin in producing arterial lesions, found cardiac lesions in a large percentage of his animals (rabbits) at an early stage of the experiment, edema and hemorrhage of heart muscle, round cell infiltration at various places, vacuolation of muscle fibers, and proliferation of connective tissue originating from the blood vessels and endocardium; at a later period an appearance of anemic infarcts or areas of induration; at the last cardiac hypertrophy supervened. Pearce examined the hearts of a number of rabbits killed immediately after an injection of adrenalin or at various intervals in the course of repeated injections of adrenalin. In the former cases he noted an edema of the heart muscle, while the latter

showed early degenerative changes of muscle fibers, followed by an increase of interstitial connective tissue. Fleischer and Loeb later repeated the experiments of Pearce. They noted that the action of adrenalin in producing myocarditic lesions is greatly increased by a previous injection of spartein, caffein or other heart tonics; the injection of a single dose of spartein or caffein (0.012 grms. of spartein sulphate or 0.025 grms. caffein sodium benzonate per kilogram) with adrenalin (0.2 cc. of a 1:1000 solution) was strong enough to cause the appearance of gross myocarditic lesions in 60 per cent. of the rabbits injected, and the appearance of microscopic lesions in almost all of the animals. Strickler and Fleischer have made a similar experiment upon dogs and observed that the injection of adrenalin plus spartein did not cause any myocarditic lesions, although it sometimes induced clinical symptoms quite similar to those occurring in rabbits as the result of the same treatment. Pisani also investigated the myocarditic lesions caused by adrenalin, and noted an early occurrence of degenerative changes of muscle-fibre (vacuolation, granular dissolution, etc.) and the later appearance of localized fibrosis originating in the interstitial tissue, which change was preceded by the infiltration of inflammatory cells. Anitschkow repeated the work of Fleischer and Loeb in an attempt to investigate more thoroughly the histogenesis of these myocarditic lesions. He observed that the myocarditic changes resulting from the injection of adrenalin plus spartein are of two types: (1) diffuse edema and its result, fibrosis; (2) inflammatory proliferation of interstitial tissue occurring in scattered circumscribed areas; fibroblasts present in the interstitial tissue polyblastic cells, derived partly from lymphocytes present in the circulating blood and partly from fixed wandering-cells in the interstitial connective tissue, and the "myocytes" of Anitschkow (of muscular origin) taking part in causing such inflammatory changes.

As a consequence of the work briefly cited above, it has been made clear that the injection of adrenalin into rabbits can cause in the heart muscle certain myocarditic lesions. From the statement of Anitschkow we are aware that such lesions histologically simulate those produced in the albino rats by thyroid administration. But since Strickler and Fleischer have failed to find the myocarditic lesions in the dogs injected with adrenalin plus

spartein, it is still an open question whether or not albino rats would respond as do rabbits to the same treatment.

I deem a decision on this point important for the solution of the problems concerning the pathogenesis of my myocarditic lesions, resulting from thyroid administration, inasmuch as it has long been an accepted theory that hyperthyroidism elicits an excess of adrenalin in the body presumably inducing an increased production of adrenalin in the suprarenal glands, as observed by Herring.

Spartein and adrenalin were intravenously injected into a number of albino rats, weighing 90 to 120 grms. First 0.001 grm. spartein sulphate per 100 grms. body weight in the solution of 1:100 was injected intravenously and 3 to 5 minutes thereafter 0.1 cc. of a 1:20,000 adrenalin solution per 100 grms. body weight. These proved to be the maximal sublethal doses that could be employed, inasmuch as four out of ten rats died immediately after the last injection, showing symptoms referable to an acute edema of lungs, i. e., dyspnea and flow of frothy, bloody fluid from the nostrils. Of seven males that survived the injection of adrenalin plus spartein five were killed by chloroform eight days and two eighteen days after the injection. For the purpose of vital staining six of them received intravenous injections of carmine (0.15 cc. of a 4 per cent. solution) daily for four days before death. Three female rats were killed without vital staining sixteen days after the injection of adrenalin and spartein.

On microscopic examination only one of the five males killed eight days after the injection of adrenalin-spartein exhibited in the heart muscle small perivascular areas of lymphocytic infiltration and proliferation of the "histiocytaire" cells. Another animal showed a slight proliferation of connective tissue in the neighborhood of the blood vessels of the heart muscle. The heart muscle of the others appeared normal. Two males killed as late as eighteen days after the injection of adrenalin-spartein showed normal heart muscle. Such was the case also in the three females killed sixteen days after the injection.

From this experiment it is evident that the injection of adrenalin plus spartein may cause certain myocarditic changes in the hearts of albino rats, but that these are far less severe than those occurring in rabbits; furthermore, that they are considerably less marked than those occurring in albino rats as the result of thyroid feeding.

(7) Anatomical Changes Observed in Other Visceral Organs of Thyroid-fed Albino Rats

In order to estimate the functional value of a heart showing the myocarditic lesions described, it was thought desirable to examine all of the other internal organs and all parts of the body, especially those which respond promptly to the impaired action of a diseased heart by certain marked anatomical changes referable to a retarded circulation of blood. Furthermore, it was thought possible that such an investigation might furnish us data that would help toward a satisfactory explanation of the mechanism through which the myocarditic lesions described are evolved.

Cyanosis of marked degree and wide extent was seen in almost all of the animals that died under the influence of thyroid feeding. On dissection many of the visceral organs, especially the lungs, liver, and kidneys, were found to be much congested and swollen, while dropsy in the pericardial, thoracic, or abdominal cavity was rarely found. Except in the lungs, there was no hemorrhagic condition in any of the visceral organs.

Liver, spleen, lungs, kidneys, suprarenal glands, and pancreas were examined microscopically. The material was obtained from fourteen normal albino rats, used as controls (ten males and four females), twelve that had been killed after having received toxic doses of thyroid, nine that were found dead in the course of thyroid administration, and ten that were killed after having received prolonged administration of non-toxic doses of thyroid. The findings in this examination will be briefly described.

Lungs.—The lungs of albino rats, even the apparently healthy controls, were frequently found infected (85 per cent.), and showing evidences of a chronic bronchopneumonia. In the majority of cases, however, the inflammatory change was not in the progressive stage, but rather in a condition of incomplete healing. The walls of the bronchi or bronchioli and those of the air-cells were thickened by the formation of large quantities of edematous young connective tissue with little inflammatory exudate. Some of such bronchi or bronchioli were found obstructed with connective tissue, often causing a secondary localized atelectasis of the related lobuli, or occasionally bronchiectasis. The lymphoid tissue present in the neighborhood of the bronchi occasionally showed a slight tendency to spread out into the adjacent parts of lung substance, and contained, besides ordinary lymphocytes, epithelioid cells, eosinophil leucocytes, plasma-cells, or fibroblasts. On vital staining with carmine some of these epithelioid cells were conspicuous from the deposit of carmine granules. In a few cases there occurred a disintegration of cell elements present in the lymphoid tissue. Evidences of acute inflammation, such as the appearance of numerous polymorphonuclear leucocytes with fluid exudate in the alveoli, were not observed in any of the control animals.

In a large percentage of the rats dying at about the end of the second week or later in the course of administration of toxic doses of thyroid, the lungs were found to be extremely congested. The capillaries in the alveolar walls were greatly dilated. Many of the alveolar epithelial cells were swollen and desquamated. Fluid matter was exuded into the air-cells, often with red blood cells.

In the rats killed at comparatively earlier periods congestion of the lungs was rarely found. In the lungs of two out of ten animals killed at an early stage of thyroid feeding, there were numerous polymorphonuclear leucocytes as well as erythrocytes which had wandered out into the alveoli in rather wide areas. It is interesting to note that in these two cases the myocarditic lesion was decidedly less severe than that found in the animals that died at later periods of thyroid feeding and which showed marked congestion in the lungs but no acute pneumonia.

It is evident, therefore, that an acute pneumonia appearing in the thyroid-fed animals has little or no part in causing the myocarditic lesions above mentioned. The same is true of chronic pneumonia, inasmuch as no myocarditic lesion was found in the control animals, although 85 per cent. of them showed a chronic bronchopneumonia.

In the rats receiving non-toxic doses of thyroid the lungs appeared little different from those of the controls.

Liver.—Frequently parasitic cysts were found in the liver of albino rats employed for these experiments. It is evident, however,

that the presence of these cysts for the most part has nothing to do with the function of the liver, for there is usually no histological change in the areas adjoining the cysts, except the rare occurrence of a slight proliferation of connective tissue. It is quite possible that the infiltration of a few small round cells about the gall ducts or blood vessels in the capsules of Glisson, found in some 36 per cent. of the normal controls, are not due to the parasitic cysts, inasmuch as such infiltrated areas were situated anywhere in the liver, quite independent of these cysts. Furthermore, there appeared to be no correlation between either the occurrence or the severity of such changes and the number of the parasitic cysts.

Marked histological changes resulting from thyroid feeding are shown in the parenchymatous degeneration, which was observed in quite a large number of animals receiving toxic doses of thyroid, whether killed at early stages in the experiment (50 per cent.) or found dead at later periods (73 per cent.). The liver cells encircling the efferent veins showed various stages of disintegration varying from simple fatty degeneration to necrosis. Occasionally such changes were present throughout the lobules, but were never entirely confined to the zone adjoining the portal veins, as observed in eclampsia gravidarum.

In addition to the parenchymatous degeneration, evidences of chronic passive congestion and its results were seen in the livers of the animals dying later in the course of the thyroid administration (71 per cent.). The efferent veins in the center of the lobules and the adjacent capillaries were greatly distended with blood. The liver cells were collapsed and occasionally even destroyed, the framework alone remaining. It was frequently seen that this framework was hypertrophied, and accompanied by marked proliferation of Kupffer's stellate cells.

On the other hand, in the peripheral zone of the lobules showing degenerative changes in the center there frequently occurred hyperplasia of the liver cells, presumably the repairing process. There appeared a large number of mitotic figures or double nuclei in the cells, and many young cells with pale and clear protoplasm and large nuclei. The occurrence of hyperplasia of liver cells without previous disintegration of the same cells was, however, very rare.

With regard to the relation existing between the various histological changes above described and the myocarditic lesions, it is of interest to note that the evidences of severe chronic passive congestion of the liver were found in the animals that showed severe myocarditic lesions; whereas in the animals showing only slight or no myocarditic lesion the liver was not congested, even when a fair degree of degenerative change was evident in the liver cells.

Spleen.—Frequently the spleen in the thyroid-fed rats was more or less congested, but not as markedly as were the lungs or the liver. There was no proliferation of reticulo-endothelial cells containing granules that stained vitally with carmine. The spleen was conspicuous for hyperplasia of the lymphoid tissue. The outlines of the Malpighian bodies became indistinct, the pulp tissue being filled with numerous lymphoid cells. Such changes were observed chiefly in the spleens examined at a comparatively late stage of thyroid feeding; i. e., in six out of nine animals, treated with non-toxic doses for a long period, four out of six animals examined in the later stage of treatment with toxic doses of thyroid, but in only one out of ten animals killed at an early stage of similar thyroid administration.

Kidneys.—In 55 per cent. of the rats found dead after having received toxic doses of thyroid for some time, the kidneys were congested, the boundary zone especially being macroscopically a dark

red. Microscopically the blood vessels were found to be distended with blood.

The parenchyma of the kidneys also was more or less affected by thyroid intoxication. In the animals receiving toxic doses of thyroid it was not infrequent that fat had lodged in the renal cells, especially in the cells of the convoluted tubules near their base in the form of small globules which stained well with Sudan III. Some of the renal cells were swollen and contained poorly staining or pycnotic nuclei. On the other hand, the regeneration of the renal cells seemed occasionally to be accelerated under the influence of thyroid administration. In the kidneys of thyroid-fed rats there was often an increase in the number of mitotic figures or double nuclei in the cells, mainly in the renal cells of the convoluted tubules. The increased deposit in the renal cells of vitally staining granules was often evident in the animals examined at an early period of thyroid feeding. Such a change may be interpreted as indicative of an increased activity of renal cells, according to Dibbelt, Arnold and others.

Suprarenal glands: In the suprarenal glands of thyroid-fed rats I have often found more or less marked congestion, but failed to observe a hemorrhagic condition. In the specimens examined for severe thyroid intoxication many nuclei of the gland cells present in the medulla appeared pycnotic.

The results of these experiments indicate that severe myocarditic lesions are associated with a chronic passive congestion of other internal organs, especially of the lungs and liver. Secondly, it is shown that as the result of thyroid feeding various internal organs other than the heart may undergo certain pathological changes, but these changes are far less marked than those of the heart; they consist chiefly in parenchymatous degeneration or proliferation and not in inflammatory proliferation of the interstitial tissue of the organs. Proliferation of "histiocytäre" cells, as seen in the heart, does not occur in any other organ except the liver, in which Kupffer cells are increased in number presumably to replace the destroyed liver cells, the change being by no means distinguishable from what may be attributed to a chronic passive congestion.

DISCUSSION

The buccal administration of thyroid, either in non-toxic doses over a long period or in toxic doses over a short period, produced a more or less marked enlargement of the heart in albino rats, the results confirming those of Hoskins (1916) and Herring (1917). I failed, however, to obtain such a large increase in size as that observed by Herring, although thyroid had been administered in widely varying doses. In this respect my results are in close agreement with those of Hoskins. The greater enlargement of the organs obtained by Herring cannot, therefore,

be attributed to the larger doses of thyroid but must have been due to some other factor.

Besides enlargement of the heart, the administration of thyroid, especially in toxic doses, produced myocarditic lesions of a chronic non-suppurative nature. Although the animals were examined at various intervals in the course of the thyroid administration, it has been quite difficult to study the sequence of the myocarditic changes step by step, inasmuch as different animals, treated with the same doses of thyroid for the same length of time, may exhibit myocarditic lesions varying not a little from one another in their histological appearance, presumably owing to a difference in the individual's susceptibility to the effect of thyroid. Nevertheless, judging from the findings in a considerable number of animals examined at various periods in the course of thyroid feeding, it seems reasonable to assume some such sequence of changes as the following.

At an early period one observes a more or less marked proliferation of clasmocytes in the interstitial connective tissue of the myocardium. These cells, conspicuous by reason of the deposit of vitally stained granules in the cytoplasm ("histiocytaire Zellen" of Kiyono), tend to accumulate densely in the small areas between the muscle-fibers or in the neighborhood of blood vessels. An irritative proliferation of these cells is suggested by the fact that some of them show mitotic figures, while others appear in the form of wandering cells with rounded-up cytoplasm. A few lymphocytes usually accompany these cells, and occasionally cell masses of comparatively small size may consist chiefly of lymphocytes. It is difficult to decide, however, whether or not lymphocytic infiltration always precedes the proliferation of the histiocytaire cells. Muscle-fibers adjoining the areas of interstitial changes may apparently be pushed aside by the pressure of the cell masses, but for a while they are kept nearly intact in their morphological appearance.

At a later period the "histiocytaire" cells increase enormously in number. Some of these cells, presumably old ones, show various changes referable to a disintegration. Fibroblasts begin to appear in greater or less numbers within the areas. Some of these exhibit the mitotic figures indicative of a rapid cell division. When the inflammatory proliferation of the interstitial tissue has advanced so far as to occupy quite wide areas, the

adjacent muscle-fibers undergo disintegration or destruction, such as vacuolization, dissolution, etc.

At the last period fibroblasts or young connective-tissue cells appear to be prevalent in the myocarditic areas, and the "histiocytäre" cells are no longer wandering, but resting fixed cells. Eventually, fibrous scars may be formed there, though unfortunately none of the specimens examined showed such.

The myocarditic areas may increase in size to such an extent as to be seen easily under the low power microscope, but never become visible to the unaided eye.

In the course of continued thyroid feeding new areas of myocarditic lesions, showing a sequence of changes such as that described above, seem to set in. Therefore, in the later stages of thyroid feeding the histological appearance of inflammatory changes varies widely in different myocarditic areas, even in the same heart.

Notwithstanding a marked inflammatory reaction of the interstitial tissue, changes of the parenchyma are in general seemingly less severe, at least in the morphological appearance observable under the microscope. The muscle-fibers may appear diffusely swollen, their cross-striations less marked than normal; on vital staining with carmine they stain diffusely pink. Occasionally, their nuclei may show various stages of disintegration, i. e., an accumulation of chromatin along the axis of the nucleus, impaired tingibility, pycnosis, etc. Such changes all are in general accord with those earlier described by Farrant in thyroid-fed rabbits. Severe parenchymatous degeneration, such as dissolution of muscle-fibers, occurs to only a limited extent in the neighborhood of quite large areas of interstitial changes.

It is interesting to note that the inflammatory proliferation of the interstitial tissue develops quite independently of the diffuse parenchymatous degeneration just described. The muscle-fibers adjacent to the small areas of interstitial changes may be pushed aside, presumably by the pressure of growing granulation tissue, but do not always show degenerative changes, although they may be severely affected later, when the granulation tissue increases to such an extent as to occupy quite wide areas. We cannot assume, therefore, that the inflammatory proliferation of interstitial tissue occurs simply to replace the degenerated muscle-fibers, as has been noted both by Mollard and Regaud,

and by Anitschkow in studying the histogenesis of myocarditic lesions produced by the injection of diphtheria toxin. The inflammatory changes in the interstitial tissue appear to be evoked primarily by the inflammatory stimulation exerted directly upon the interstitium by some toxic agent, which may also be responsible for the occurrence of diffuse parenchymatous degeneration.

There has been an accepted theory that hyperthyroidism elicits an excess of adrenalin in the body, presumably inducing an increased production of adrenalin in the suprarenal glands (Herring); and, as cited before, Anitschkow has observed in rabbits, after the injection of adrenalin plus spartein, an inflammatory proliferation of the interstitial tissue of the myocardium, not always associated with degenerative changes in the muscle-fibers, and he ascribed such changes to the effect directly exerted by these chemical substances upon the interstitial tissue. It is worth while to discuss whether or not the myocarditic lesions in hyperthyroidism are due to the excess of adrenalin in the body. It is evident, however, that this probability is not of importance, for I am aware, from chemical examination, that the production of adrenalin in the suprarenals is liable to be interfered with by the administration of thyroid in such doses as to cause marked myocarditic lesions (0.5 grms. desiccated thyroid per day), and, from histological examination, that evidence of disintegration of gland-cells in the medulla of the suprarenals may occasionally accompany the myocarditic lesions. Furthermore, I have ascertained that the heart of the albino rat does not respond to injected adrenalin with so marked inflammatory changes as have been observed in rabbits by K. Ziegler (1905), Fleischer and Loeb (1909-10), Anitschkow (1913), etc.

It is generally known that various acute infections—thyroid bacillus (Romberg), meningococcus and streptococcus (Gruber)—may cause myocarditic lesions with an acute inflammatory proliferation of the interstitial tissue. In my thyroid-fed animals the marked myocarditic lesions were not associated with evidences of acute infection observable microscopically in the other parts of the body. As noted already, it has been proved that the presence of a pneumonic affection in the lungs or of parasitic cysts in the liver is of little significance in causing myocarditic lesions.

As is generally known, hyperthyroidism necessitates an over-exertion of the heart, a rapid pulse being evident. Nevertheless, the myocarditic lesions herein described may not be due solely to an excessive mechanical strain, inasmuch as the animals forced to overexertion in a high temperature did not exhibit the same myocarditic lesions as those occurring as the result of thyroid feeding.

Considering all the evidence, I am inclined to believe that the stimulative or toxic agent responsible for the various degenerative and inflammatory changes in the myocardium might be found in the thyroid substance itself, introduced into the body in excessive amounts by thyroid administration.

We find it difficult, however, to explain the fact that the heart usually is affected far more severely than other visceral organs. There is the possibility that mechanical strain or over-exertion of the heart may render the myocardium less tolerant to thyroid intoxication (*locus minoris resistentiae*), and thus facilitate the occurrence of myocarditic lesions, though a mechanical strain is not of itself sufficient to produce such lesions.

It is likewise difficult to explain the fact that the inflammatory changes occurring in the myocardium as the result of thyroid administration seem to be remarkable for an irritative proliferation of the "histiocytaire" cells present in the interstitial connective tissue. We are not justified in at once assuming that the inflammatory stimulus exerted by the thyroid substance is conspicuous from a histiotactic character. If the stimulus eliciting the proliferation of the "histiocytaire" elements were characteristic of the effect exerted by thyroid substance, the other visceral organs should show a similar proliferation. So far as I am aware, however, such is not the case. There is a hyperplasia of Kupffer's cells in the liver around the efferent veins, where the gland cells are annihilated, but this is by no means distinguishable from what occurs as the result of a chronic passive congestion. On the other hand, in the spleen there appears to be a proliferation of the lymphoid tissue, whereas the reticulo-endothelial cells, "histiocytaire" cells, are apparently not increased in number.

As to the functioning power of a heart showing the myocarditic lesions described, it is quite certain that it is distinctly inferior to that of the normal heart. It is of interest to note here

that the most severe myocarditic lesion was found in a thyroid-fed rat that died suddenly after overexertion, presumably as a result of an acute cardiac insufficiency. On histological examination of the other internal organs of a large number of thyroid-fed animals, it has been further ascertained that the severe myocarditic lesions are for the most part associated with changes referable to a generally retarded circulation of blood—a chronic passive congestion of various organs and its results. It is highly probable, however, that an inflammatory proliferation of the interstitial tissue may be of little significance in causing the cardiac insufficiency, inasmuch as the muscle-fibers are apparently not directly destroyed to any great extent by such changes. It seems likely that the cardiac insufficiency is due rather to the diffuse degenerative changes occurring at the same time in the muscle-fibers. Since a large number of muscle-fibers are injured, even though slightly, it is a matter of course that such hearts are not able to meet successfully a demand for extra work.

In comparing the myocarditic lesions resulting from thyroid intoxication with those occurring in the myocardium of human subjects suffering from an acute articular rheumatism, as Aschoff (1904) and Tawara, Geipel (1905), Bracht and Wächter (1909), Fraenkel (1912) and many others have described, we are struck by the similarity of the changes in these two sets of the conditions, especially in the fact that they both appear as periarterial nodules composed of large, pale, peculiar cells.

Concerning the etiology of the rheumatic myocarditis, nothing certain is known. Bracht and Wächter produced in rabbits by the inoculation of the diplostreptococcus obtained from two patients suffering from an acute articular rheumatism, not only swelling of the arterioles and valvular affection of the heart, but also myocarditic lesions with degenerative changes of muscle-fibers, lymphoid cell infiltration and fibroblast proliferation, the changes corresponding in general to those in rheumatic patients, except that they did not appear as periarterial nodules. As to the cause of the nodular changes appearing in the heart of patients suffering from rheumatism, these investigators presumed that the causative bacteria might be accumulated in the neighborhood of blood vessels or between the muscle-fibres and elicit a periarterial infiltration of lymphocytes, by the action of which cells the bacteria are annihilated; and that the toxic sub-

stances produced by bacterial disintegration might evoke the formation of the nodules, which are composed of cells derived from connective tissue cells,—the changes being regarded as referable to the healing process. De Vecchi (1912), however, successfully produced myocarditic lesions localized in periarterial areas, by the injection of sterile blood obtained from a patient suffering from articular rheumatism. Aschoff contended that the nodular formations consisting of large pale cells are specific for rheumatic myocarditis, and many other investigators have confirmed his opinion. Geipel alone observed similar lesions in the heart of a patient with the contracted kidneys, and he assumed that other toxins or infective agents also may cause myocarditic changes similar to those of the articular rheumatism. It is significant that such changes can also be produced in the myocardium of animals by the buccal administration of thyroid.

In regard to the origin of the large peculiar cells composing the nodular formations of the rheumatic myocarditis, there is a considerable divergence of opinion. Aschoff and Tawara at first termed these cells "abnormally large leucocytoid elements," whereas Geipel regarded them as being derived from the connective tissue cells. Saigo was inclined to assume that some of them are of muscular origin. Bracht and Wächter agreed with Geipel in considering these cells as derived from the connective tissue cells, the fibroblasts. Aschoff (1919) stated briefly in his text-book of pathology, published later, that these cells take their origin from the connective tissue. In my observations on myocarditic changes resulting from thyroid intoxication I have ascertained, by the aid of a vital staining with carmine, that the large peculiar cells composing the myocarditic patches are derived from clasmocytes present in the interstitial connective tissue, the "histiocytaire" cells of Kiyono.

The question now arises, How do the nodular formations develop in these cases in which no bacteria take part, as supposed by Bracht and Wächter? So far as I know, in the myocarditic process resulting from thyroid feeding there is an irritative hyperplasia of the clasmatocytes in the interstitial connective tissue. The clasmatocytes primarily are not distributed uniformly throughout the interstitial tissue of the myocardium, even in normal hearts, but show a tendency to cluster in the sheaths of the blood vessels and in the areolar tissue beneath the

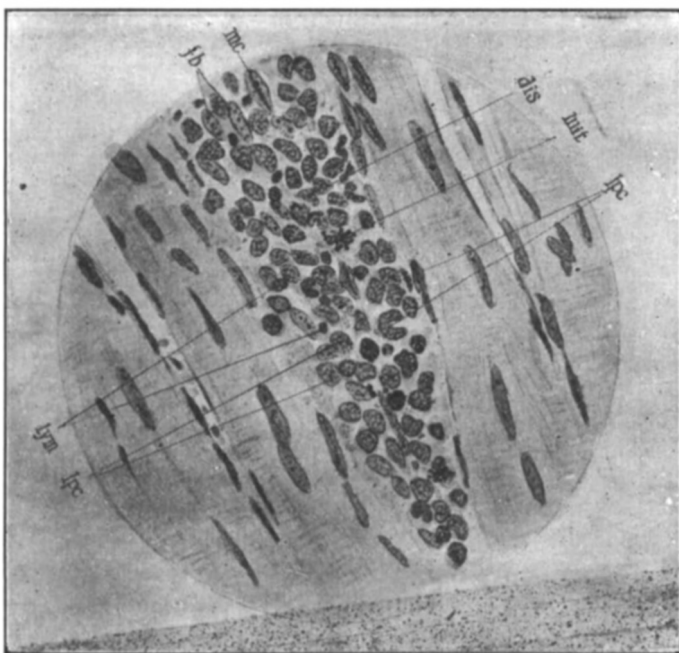
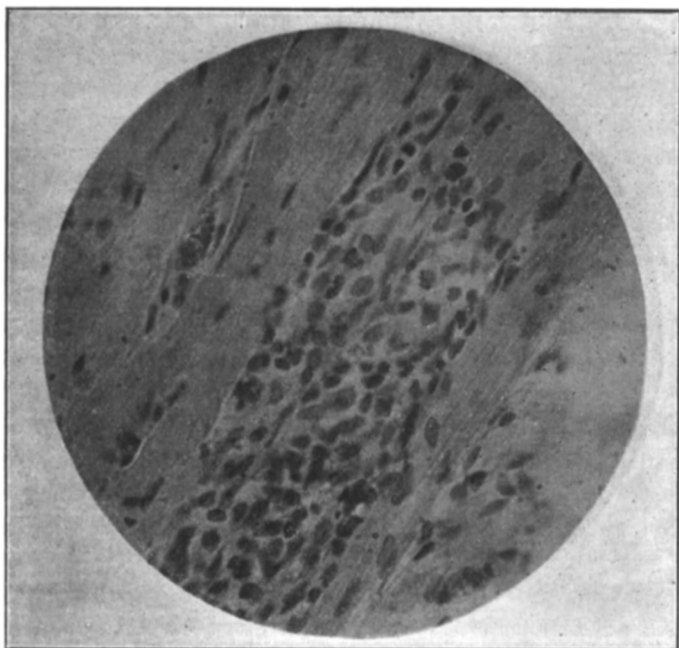
pericardium or beneath the endocardium. If there may set in an inflammatory proliferation of these cells, the histiocyte cells derived from them would, as a matter of course, tend to accumulate in periarterial or sub-pericardial circumscribed areas, inasmuch as their mother-cells exist in these sites in larger numbers than elsewhere. In view of such an explanation we might assume that any inflammatory process in which the "histiocyte" cells are caused to proliferate should be able to give rise the nodular formations consisting chiefly of large, pale, peculiar cells, and showing a tendency to form in the neighborhood of the blood vessels,—the microscopic appearance of these corresponding nearly with what has been described by Aschoff as specific and unique for the rheumatic myocarditis.

Bircher has produced goitrous changes in the thyroid glands of rats and rabbits by giving the animals water from certain wells alleged to produce goitres. According to his statement, tachycardia and hypertrophy of the heart were evident in these goitrous animals, and furthermore the heart-muscle showed a cloudy swelling or fatty degeneration which was associated with leucocytic infiltration and later scar formation. Comparing such myocarditic changes with those resulting from thyroid administration we find difficulty in discussing them in detail, as Bircher's statement concerning the histology of the affected heart-muscle is quite brief. What types of cells did the leucocytic infiltration consist of? This is a point to be established before discussing the relation of the changes.

In conclusion I will compare the myocarditic lesions resulting from thyroid administration with those found by Fahr in human goitre hearts. My findings are in general agreement with those of Fahr in the following points. (1) The inflammatory processes are chiefly localized in the interstitial tissue, the parenchymatous changes being less marked; (2) the inflammatory change is of a non-suppurative nature; polymorphonuclear leucocytes are present but sparsely in the inflammatory areas; (3) a tendency is shown to form cell collections between the muscle-fibres or in the neighborhood of the blood vessels. Concerning the constituents of the cell masses, Fahr stated that they consisted chiefly of lymphocytes, occasionally accompanied by fibroblasts. I myself have observed microscopically in the heart muscle of an exophthalmic goitre patient (a woman of 25 years.

who died immediately after a partial thyroidectomy without any further complications), in small periarterial areas, a number of small cells of lymphoid type but no cells of large mononuclear type. The muscle-fibres show in addition great quantities of a yellowish brown, granular pigment, slightly stainable with Sudan III. In another case (a woman aged 48 years, who died immediately after a subcutaneous injection of a small dose of adrenalin, without other complications) there were similar lymphocytic infiltrations between the heart-muscle fibres in the neighborhood of blood vessels or beneath the pericardium. In this case the small round cells of lymphoid type were accompanied by a quite large number of large pale cells—simulating the large mononuclear or transitional cells present in the circulating blood—and also a number of fibroblasts or myocytes. Myocytes were increased in number and diffused throughout the interstitial tissue of heart muscle. As has been repeatedly stated above, the myocarditic changes resulting from thyroid intoxication show large pale cells as their chief constituents, though in some cases small round cells of lymphoid type may compose mainly the myocarditic formations. And it has been further ascertained that most of the large pale cells correspond with the “histiocytaire” cells of Kiyono, the cells derived from clasmatoocytes or fixed wandering cells present in the connective tissue. No vital staining being possible in human subjects, it is hardly possible to determine how large a proportion of the cells present in the myocarditic areas of human goitre hearts are of the “histiocytaire” type, derived from the fixed wandering cells. According to Maximow, however, lymphocytes as well as “histiocytaire” cells, might be included among the polyblastic wandering cells. If we employ Maximow’s terms, *polyblastic* cells, instead of *lymphoid* or *histiocytaire* cells, as Anitschkow has done in describing the myocarditic changes, we may identify such changes resulting from thyroid intoxication with those of human goitre hearts in this respect.

Upon the basis of the anatomical findings Fahr concluded that the toxic agent circulating in the body of the goitre patient acts directly upon the heart muscle. Judging from the fact, observed in my experiments, that a mechanical strain or over-exertion of the heart of itself cannot cause myocarditic lesions, as does thyroid administration, we might be justified in assum-



One of the myocarditic areas, found in the heart-muscle of a rat, which died suddenly on the twentieth experimental day in the course of the daily administration of 0.5 grms. desiccated thyroid per day. The collection of cells, situated between the muscle-fibres, consist chiefly of large pale cells (lpc)—mainly histiocytic cells derived from clasmocytes—, associated with smaller numbers of small round cells—microlymphocytes (lym)—, fibroblasts (fb) or myocytes (mc). Two mitotic figures (mit) and some more or less dis-integrated nuclei (dis) are seen.

ing that the thyroid substance, introduced in large amounts into the animal organism, or an excess of thyroid secretion, produces not only an excessive stimulation of the sympathetic nerves, thus causing an increased rate and force of the heart beat, but also a toxic effect upon the heart muscle itself, causing various degenerative and inflammatory changes associated with the functional insufficiency.

The results of my experimental investigations do not furnish us with the data which would enable us to state with certainty that the cardiac disturbances associated with endemic goitres have nothing to do with general intoxication, presumed by Bircher to be responsible for the changes in the thyroid glands as well as those in the heart, or to decide positively, whether or not there are any qualitative alterations in the thyroid secretion which take part in causing the cardiac changes associated with Graves' disease. I believe, however, that my results have thrown further light upon the etiology of goitre heart, and establish evidence that cardiac disturbances, almost identical in every respect to those observable in goitrous patients, can be produced by thyroid intoxication or by an excess of thyroid secretion circulating in the body.

SUMMARY AND CONCLUSION

1. The buccal administration of toxic doses of thyroid caused, in addition to enlargement of the heart, the appearance of myocarditic lesions in a large percentage of albino rats: in 90 per cent. of the animals killed during the first and second week and almost all of those dying in the second or third week of a daily administration of 0.5 grms. desiccated thyroid.

2. The myocarditic lesions consisted chiefly in dense accumulations of large "histiocytäre" cells (Kiyono), derived from the clasmocytes present in the interstitial connective tissue, in small circumscribed areas between muscle-fibres or not infrequently in the neighborhood of the blood vessels. These cells may be accompanied by a small or occasionally rather large number of cells of lymphoid type, at earlier periods in the sequence of the myocarditic changes; in the later stages they may be associated with fibroblasts, increasing gradually in number, and eventually prevailing over the other types of cells. The muscle-fibres may be destroyed in confined areas adjoining larger areas

of myocarditic changes. The muscle-fibres may show, moreover, slight but diffuse degenerative changes, apparently occurring independent of the interstitial changes described above, such as indistinctness of cross-striations, slight tingibility upon vital staining with carmine, or slight disintegration of the nuclei.

3. The interstitial inflammatory proliferation and the diffuse parenchymatous degeneration described above may both be attributed directly to thyroid intoxication.

4. The hearts showing such myocarditic lesions are functionally inferior to normal hearts.

5. In their histological appearance the myocarditic lesions resulting from thyroid administration are closely related to those observed by Aschoff, Tawara and many others in the hearts of individuals suffering from rheumatism.

6. The myocarditic lesions occurring in experimental hyperthyroidism induced by thyroid administration correspond to those in goitre hearts, first noted by Fahr, in the sense that they both consist of a chronic non-suppurative interstitial myocarditis. It is evident, therefore, that thyroid administration can cause not only tachycardia or hypertrophy, but also myocarditic lesions, all of which simulate the functional and anatomical changes found in human goitre hearts.

7. This evidence lends support to the theory that the cardiac disturbances associated with goitre are due to thyroid intoxication, and further, to an excess of thyroid secretion.

The experimental work has been done in the laboratory of Professor K. Miura's medical clinic. I am much indebted to him for his care and supervision.

BIBLIOGRAPHY

1. Anitschkow (N.): Experimentelle Untersuchungen über die Neubildung des Granulationsgewebes im Herzmuskel. Beitr. z. path. Anat. u. z. allg. Path. (Jena), 1913, **55**, 373-415.
2. Anitschkow (N.): Ueber die Histogenese der Myocardveränderung bei einigen Intoxikationen. Virchow's Arch. f. path. Anat. (etc.) (Berl.), 1913, **211**, 193-232.
3. Aschoff (L.): Zur Myocarditisfrage. Verhandl. d. deutsch. path. Gesellsch. (Jena), 1904, **8**, 46-53.
4. Aschoff (L.): Pathologische Anatomie, 1919, IV, Aufl. II, 39.
5. Bircher (E.): Neuere Arbeiten auf dem Gebiete der Hemophilie. Med. Klin. (Berl.), 1910, **6**, 544-596.
6. Bircher (E.): Weitere histologische Befunde bei durch Wasser erzeugten Rattenstrumen und Kropfherzen. Deutsche Ztschr. f. Chir. (Leipz.), 1911, **112**, 368-424.
7. Bracht (E.) & Wächter: Beitrag zur Aetiologie und pathologischen Anatomie der Myocarditis rheumatica. Deutsches Arch. f. klin. Med. (Leipz.), 1909, **96**, 493-514.
8. Donaldson (H. H.): The Rat. Memoirs of the Wistar Institute of Anatomy and Biology (Phila.), 1915, No. 5.

8. Fahr (T.): Histologische Befunde an Kropfherzen. *Centralbl. f. allg. Path. u. path. Anat. (Jena)*, 1916, **27**, 1-5.
9. Farranti (R.): Hyperthyroidism: its experimental production in animals. *Brit. M. J. (Lond.)*, 1913, **II**, 1363-1367.
10. Fleischer (M. S.) & Loeb (L.): Experimental myocarditis. *Arch. Int. Med. (Chicago)*, 1909, **3**, 78-91.
11. Fraenkel: *Beitr. z. path. Anat. u. z. Allg. Path. (Jena)*, 1912, **52**, 597.
12. Von Fürth & Schwarz (K.): Ueber die Einwirkung des Jodothyryns auf den Zirkulationsapparat. *Arch. f. d. ges. Physiol. (Bonn)*, 1908, **124**, 113-156.
13. Geipel (P.): Untersuchungen über rheumatische Myokarditis. *Deutsches Arch. f. klin. Med. (Leipz.)*, 1905, **85**, 75-88.
14. Herring (P. T.): The action of thyroid upon the growth of the body and organs of the white rat. *Quart. J. Exper. Physiol. (Lond.)*, 1917, **11**, 231-254.
15. Herring (P. T.): The effects of thyroidectomy and thyroid feeding upon the adrenalin content of the suprarenals. *Quart. J. Exper. Physiol. (Lond.)*, 1916, **9**, 391-401.
- Herring (P. T.): The effects of thyroid feeding on the weight of the suprarenals and their adrenaline content. *Quart. J. Exper. Physiol. (Lond.)*, 1917, **11**, 47-58.
- Herring (P. T.): The adrenalin content of the suprarenals of the female white rat, and changes in it brought about by thyroid feeding and other conditions. *Quart. J. Exper. Physiol. (Lond.)*, 1919, **12**, 115-124.
16. Hewitt (J. A.): The effect of administration of small amounts of thyroid gland on the size and weight of certain organs in the male white rat. *Quart. J. Exper. Physiol. (Lond.)*, 1920, **12**, 347-354.
17. Hoskins (E. R.): The growth of the body and organs of the albino rat affected by feeding various ductless glands (thyroid, thymus, hypophysis, pineal). *J. Exper. Zool. (Phila.)*, 1916, **21**, 295-346.
18. Iscovesco (H.): Action physiologique, en particulier sur la croissance d'un liquide (IIBa), extrait de la thyroïde. *Compt. rend. Soc. de Biol. (Paris)*, 1913, **75**, 361-363.
19. Kiyono (K.) & Tanaka (Y.): Ueber die vitale Färbung der Knochensubstanz. *Kyoto Igaku Zasshi*, 1917, **14**, 76.
- Kiyono (K.): Die vitale Karminspeicherung. *Jena*, 1914, 265, p. 5, pl. 8°.
20. Kuriyama (S.): The influence of thyroid feeding upon carbohydrate metabolism. III. The acidosis in experimental hyperthyroidism and its relation to epinephrine in the blood and the decrease of liver glycogen. *J. Biol. Chem. (N. Y.)*, 1918, **33**, 215-227.
21. Litten: Virchow's Arch. f. path. Anat. (etc.) (Jena), 1887, **70**, 10.
22. Masuda (N.): Untersuchungen über die Zellfunction mit Hilfe der vitalen Färbung. *Ztschr. f. exper. Path. u. Therap. (Berl.)*, 1911, **9**, 250-267.
23. Mollard (J.) & Regaud (C.): Lesions chroniques expérimentales du myocarde consécutives à l'intoxication diphthérique. *Compt. rend. Soc. Biol. (Paris)*, 1897, **10**, s. IV. 674-755.
24. Nasaroff: Einige Versuche über künstliche Abkühlung und Erwärmung warmblütiger Thiere. *Virchow's Arch. f. path. Anat. (etc.) (Jena)*, 1882, **90**, 482-499.
25. Naunyn (B.): Kritisches und experimentelles zur Lehre vom Fieber und von der Kaltwasserbehandlung. *Arch. f. exper. Path. u. Pharmacol. (Leipz.)*, 1884, **18**, 49-128.
26. Pearce (R. M.): Experimental myocarditis: a study of the histological changes following intravenous injections of adrenaline. *J. Exper. Med. (N. Y.)*, 1906, **8**, 400-409, 1 pl.
27. Simmonds (M.): Ueber die anatomischen Befunde bei Morbus Basedowii. *Deutsche med. Wchnschr. (Leipz. u. Berl.)*, 1911, **37**, 2164-2168.
28. Stadler (E.): Experimentelle und histologische Beiträge zur Herzhypertrophie. *Deutsches Arch. f. klin. Med. (Leipz.)*, 1907, **91**, 98-127.
29. Strickler (A.) & Fleischer (M. S.): The influence of intravenous injections of sparteine and adrenaline on the heart of the dog. *J. Pharm. & Exper. Therap. (Balt.)*, 1910, **2**, 55-57.
30. de Vecchi (B.): Sur la myocardite rhumatismale; étude anatomopathologique et expérimentale. *Arch. de méd. exper. et d'anat. path. (Paris)*, 1912, **24**, 352-420, 1 pl.
31. Werhovsky (B.): Untersuchungen über die Wirkung erhöhter Eigenwärme auf den Organismus. *Beitr. z. path. Anat. u. z. allg. Path. (Jena)*, 1895, **18**, 72-114, 1 pl.
32. Ziegler (K.): Ueber die Wirkung intravenöser Adrenalininjektion auf das Gefäßsystem und ihre Beziehung zur Arteriosklerose. *Beitr. z. path. Anat. u. z. allg. Path. (Jena)*, 1905, **38**, 229-254.