STUDIES ON THE PAROTID AND THYROID GLANDS OF CARIES-SUSCEPTIBLE AND CARIES-RESISTANT STRAINS OF RATS (*RATTUS NORVEGICUS*, BERKENHAUT)

By

Roger F. Keller, Jr.

A THESIS

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Department of Zoology

1953

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AN ABSTRACT

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Approved Harriman R. Hunt

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Abstract

Hunt and Hoppert (1944) produced two strains of albino rats, one of which is extremely resistant to dental caries in the molar teeth and the other of which is susceptible to dental caries. This differentiation was accomplished by progeny testing, close inbreeding and the use of a caries-producing diet. The present researches were conducted to ascertain whether these hereditary differences are due to either the secretions of the parotid gland or to the function of the thyroid gland.

The parotid duct was severed and a section of it removed bilaterally from 35- to 40-day-old rats of both strains. These rats were then observed at bi-weekly intervals for the appearance of macroscopic dental caries. The experimental procedure did not markedly alter the caries production of either strain of rats and the gross differences between the strains of rats persisted. It was concluded that the secretions of the parotid gland are not of great importance in the caries process of these animals.

The thyroid glands of both strains of rats were examined in histological sections from tissues which were prepared with Bouin's fixative, mounted in paraffin, cut at seven microns thickness and stained with hematoxylin and eosin. It was found that caries-susceptible rats had larger colloid areas and that the mean height of their acinar epithelium was less than that of the caries-resistant rats.

It was observed that the thyroid glands of the rats in the susceptible series were about 43 percent heavier (on a mg. per 100 gm. body weight basis) than were the glands of the resistant series.

There was shown to be a 42 percent faster turnover of intraperitoneally injected radioactive iodine in the thyroid glands of the resistant strain than in the susceptible strain. There were no demonstrable differences in oxygen consumption when the two strains were compared in the closed circuit type of unit. Therefore, it may be assumed that the thyroid secretion rate is about the same in both strains of rats. It would appear that there is a compensatory mechanism in the susceptible strain's thyroid glands so that a large gland which has the appearance of an underactive gland, and which has a slower turnover of iodine, actually is metabolically equivalent to that of the smaller, more active gland of the resistant series.

The use of thyroidally active protamone which raises the metabolic level and thiouracil, a goitrogen, failed to significantly alter the production of dental caries in the susceptible series rats.

Inbreeding tends to produce stocks which differ from one another in a number of traits. Each stock may approach homozygosity for the traits which characterize it. The diverse traits in a stock are associated as a consequence of the chance recombination of genes in inbreeding, one trait need not be a cause of another one. It would appear that the thyroid traits

in these rats are associated with susceptibility or resistance to caries but that the former are not the cause of the latter.

Hunt, H. R. and C. A. Hoppert. Inheritance of susceptibility and resistance to caries in albino rats (Mus norvegicus). J. Am. Col. Dentists. 11: 33-37, 1944.

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Dissertation: Studies on the Parotid and Thyroid Glands of Caries-Susceptible and Caries-Resistant Strains of Rats (Rattus norvegicus, Berkenhaut)

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I. INTRODUCTION

Hunt and Hoppert (1944) proved that heredity is an important factor in the etiology of dental decay in the albino rat. This was accomplished by producing two decidedly different strains of rats, one of which is susceptible to dental caries in the lower molar teeth and the other which is very resistant. This differentiation was brought about by phenotypic selection, progeny testing, close inbreeding and the use of a caries producing diet devised by Hoppert, Webber and Caniff (1932).

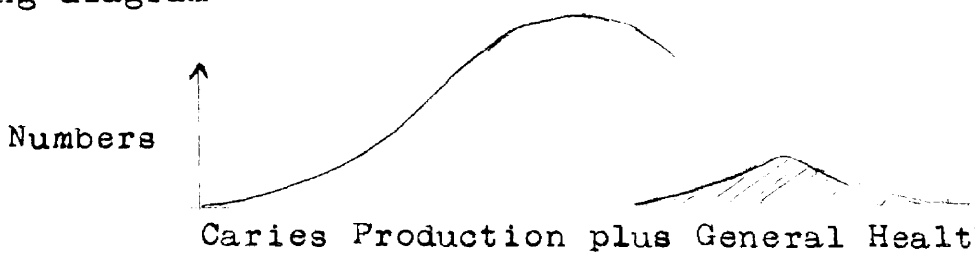
Continued selection and brother-sister inbreeding since the initiation of this project in 1937 increased the differences in resistance to dental caries between these two strains of rats. The studies described in this paper were made on the seventeenth generation of the caries-resistant series and the twentieth generation of the caries-susceptible rats of these strains.

One of the objectives in the project of Hunt and Hoppert was to determine the mechanisms of the inheritance of these hereditary differences which were isolated. Before this may be determined, however, the physical and physiological factors which are involved in the hereditary aspects of dental caries must be determined. It was with this view in mind that the present researches were conducted.

The Selection Which Has Been Practiced

Phenotypic and genotypic selection have been practiced to produce caries-susceptible and caries-resistant lines of rats. Those animals which developed caries soonest in the first generation were mated with each other, then the most susceptible progeny of such crosses were mated by the brother x sister system. Thereafter the most susceptible animals within each sibship were mated with each other. The most resistant rats in the first generation were crossed with one another, then the most resistant animals within each sibship were mated together. By brother x sister matings a resistant strain was created. The performance of an animal was not an adequate index of the genes it carried (Hunt, Hoppert, and Erwin, 1944). Breeders were selected, not only on the basis of the time required to produce dental caries in themselves, but also according to the average time at which caries appeared in the sibship to which they belonged. The caries records of the females and their siblings were the only criteria for selecting female breeders. The weight of a prospective male breeder at 70 days of age was also considered, the heaviest male with the best caries record being used.

Such a process of selection may be illustrated by the following diagram:



The entire population is represented by the area bounded by the larger curve and the animals selected for breeding by the shaded area.

This situation would, in general, be true for each generation and for each strain of rats.

Is Each Line a Homozygous Population?

Inbreeding tends to produce homozygosity with respect to all the genes in a population. One might think that every family within the resistant (or susceptible) series would be homozygous for the same genes. Intense selection for resistance (or susceptibility) might be expected to lead to such a result. It seems likely, however, that different families within the resistant strain are not homozygous with respect to alleles responsible for resistance. The resistants still show great variability, which decidedly discounts the notion that the stock is homozygous. On the other hand, the relative uniformity of the susceptibles suggests that there is a high degree of homozygosity within it.

The possible contributions of dominance, epistasis, linkage, and their relations to the environment in these experimental animals have not been explored. The environment has been maintained as uniform as possible. However, inasmuch as the genetic factors may be varied, their respective interactions with the environment may vary also.

The fact that selection is practised might very well be contributing to heterozygosity. This would be true if any of

the several criteria on which selection is based were due, even in part, to heterosis. Thus the heterozygous individuals produced would be selected for parents of more heterozygous individuals and the less desirable homozygotes produced would be discarded (Haldane, 1936).

Linkage could also be contributing to other forces which prevent a complete homozygosity of these two lines. The desired genes might conceivably be located close to undesired genes on the same chromosome and thus be discarded from the genic pool in the stocks. The problem is, of course, considerably more complicated than simple mono-factorial inheritance. Obviously multiple factors are involved. The genetics of such a problem must be analyzed from a biometrical point of view in order to utilize fully the tremendous wealth of information in the data. Such a task has not been undertaken as yet on a sufficiently extensive scale.

Genic Fixation Within the Lines

The method of selection of breeders and the breeding program being used may be contributing to the lack of uniform resistance to dental caries shown by the resistant line. It is undoubtedly true that in many cases desired genes have been discarded from the "genic pool" of these rats along with undesired genes.

The mating of related individuals is, of course, the only known method of producing homozygosity for desired genic

characters where polygenes are present. The more intense the inbreeding, the more rapid is the approach to the genic fixation in the homozygous state. In the work of Hunt and Hoppert, however, it has been the purpose to produce the two lines of albino rats, one susceptible and the second resistant to dental caries. This has been very successful. This isolation of the desired genic conditions has been accomplished by using rigid selection in combination with intense inbreeding. Perhaps if the inbreeding had been less intense the approach to genic fixation would have been less rapid. A slow approach has the advantage that the genic complements present may be examined before they are discarded or kept in the "genic pool" represented by the parental stocks used for the next generation. In a breeding system using less intense inbreeding and thus producing a slow fixation there is less likelihood that undesired genes would be fixed along with desired genes.

It might prove desirable also to cross some of the present lines and thus obtain recombinations of desired genes. These new combinations could again be intensified and made homozygous if desired. The probability exists that each of the lines contain some genes which may be lacking in other lines, and that such recombinations as suggested would prove to be better than any of the present lines.

II. ELIMINATION OF THE SECRETION OF THE PAROTID GLAND AND ITS RELATION TO DENTAL CARIES

Causes of Dental Caries

Caries is defined in Webster's New International Dictionary as "... the decay of animal tissues, especially of bony tissue ...". Dental caries is defined as "...localized destruction of tooth tissues by micro-organisms...". The classical theory of the cause of dental caries is the so-called acid theory of Miller (1890), who contended that acids produced by oral micro-organisms acting on dietary carbohydrate particles in the oral cavity dissolved the mineral salts from the teeth. It was also thought that proteolytic enzymes contributed to the process by dissolving the organic matrix of the teeth.

A large proportion of the dental research since Miller's original hypothesis has been devoted to the removal of the mouth acids by various means. Bunting, Nickerson, and Hard (1927) observed that the bacterium Bacillus acidophilus[1] was found in the mouths of persons having dental caries, that the presence of these bacteria tended to be localized in areas undergoing active caries, and that they were absent, or present in small numbers, where no active caries were found.

Hawkins (1929) concluded that bacteria were able to break down carbohydrates, forming a concentration of acid sufficient to decalcify teeth.

---

[1] Now known as Lactobacillus acidophilus

In summarizing a number of studies from his laboratory, Jay (1938) concluded that there existed a diagnostic relationship between oral lactobacilli and the incidence of dental caries.

Neuwirth and Klosterman (1940) demonstrated that there was a rapid production of lactic acid in the mouth when carbohydrates were allowed to ferment there. It was demonstrated that this acid formation was probably due to the action of oral microorganisms.

Several other microorganisms have also been isolated from mouths and many of these have been considered as factors in the production of acids or of enzymes in the saliva. Yeast-like organisms, (*Candida albicans*), were isolated from mouths by Lilienthal (1950), who observed a synergistic relationship between the yeast and lactobacilli which was responsible for more than normal amounts of lactic acid. Neither organism alone was able to produce the amount of acid which the two together generated.

Streptococci were regarded by Bibby *et al*. (1942) as important acid producers in the mouth and Shiere, Georgi and Ireland (1951) observed that streptocci were able to convert fermentable sugars to lactic acid.

Boyd, Cheyne and Wessels (1949) confirmed a very common observation that the lactobacillus count increases with mounting caries activity. They found, however, that the high *L. acidophilus* counts were not necessarily a cause of dental caries *per se* , but that they might simply be an accompanying

phenomenon. Hugill and Box (1950) were able to produce experimental caries in the absence of bacterial activity, and the carious lesions resembled the dental pathological lesion. Hartles and MacDonald (1950) conclude that acid production *per se* is evidently not a sufficient cause for dental caries observed *in vivo*.

A new concept of the dental caries mechanism has been advanced by Csernyei (1950). Dental lymph, with its content of phosphatase, dissolved magnesium and fluorine, passes through the ultracapillaries of the tooth into the enamel. As soon as the physiological equilibrium between the dissolved magnesium and fluorine is altered, the phosphatase may hydrolyze enamel and produce a carious lesion. The carious lesion is the first clinical sign of dental caries and would be initiated inside the tooth rather than on the outside, as the acid theory demands.

The post-eruptive tooth has recently been shown to be more active metabolically than was first thought. It has been known for some time that there was an appreciable ionic exchange between bone and the circulating fluids. Manley and Bale (1939) observed that rat molars showed a slow, but measurable turnover of phosphorus. The turnover was found to be in the inorganic portion of the molars, and this finding invalidated the concept that there is no metabolism in teeth subsequent to the initial deposition of the inorganic salts.

It has been shown that there is a metabolic relationship between the saliva and teeth with reference to calcium,

phosphorus, proteolytic enzymes as well as acids in the mouth. Dentay and Rae (1949) demonstrated that the saliva after filtering contains no phosphatase. This enzyme then seems to come from cellular debris and from oral microorganisms. Thus, the importance of the phosphatase enzyme in the saliva is being recognized as at least a component in the complex etiology of dental caries.

The Role of Salivary Secretions

The role of salivary secretions is a difficult one to determine accurately. It has been shown that desalivation of rats hastened the onset of dental caries, and that the severity was also increased. Ginn and Volker (1941) desalivated a group of rats and compared them with "sham-surgery" controls and intact controls. The results of the sham-surgery, in which all procedures were duplicated except that the salivary glands were not removed, were the same as for the intact controls.

The existence of an endocrine-like relationship between the salivary glands and the sex organs is indicated by the work of Higashizo (1941), who found that the removal of the submaxillary glands and ligation of the parotid duct in young rats resulted in hypertrophy of the uterus and atrophy of the testes.

Hukusima (1941) removed both the parotid and submaxillary glands of young rats and markedly increased the incidence of molar caries.

Kite, Shaw and Sogannes (1950) tube fed both normal and desalivated rats. Their results showed that tooth decay is prevented in caries susceptible rats when the direct effects of food in the oral cavity are eliminated. They confirmed the fact that the salivary secretions are important in the etiology of tooth decay. Weisberger, Nelson and Boyle (1940) observed that the acceleration of dental caries activity following the extirpation of the salivary glands was due primarily to changes in the cementum of the teeth.

Cheyne (1939b) removed various combinations of the major salivary glands of the rat. The greatest amount of caries followed the removal of the parotid and the submaxillary glands. The extirpation of the parotids alone produced only slightly more dental caries than in controls.

Manhold and Manhold (1949) observed a significant correlation between psychological factors and dental decay. Presumably excessive amounts of saliva would have the effect of washing the teeth, neutralizing acids, and thus reducing dental caries.

An unidentified factor in some salivas was reported by Hill (1939). There was a variation in intensity of this factor and its presence or absence was found to be consistent with the presence or absence of dental caries in rats. Subsequent investigations have not identified this factor. Hill (1953) was not able to determine any great differences between any inhibitory fractions in the susceptible and resistant strains of the Hunt-Hoppert rats.

The Relationship of Diet to Tooth Decay

The deficiency of necessary dietary elements during the period of tooth formation may have a deleterious influence on deciduous tooth formation and structure (Howe, 1923). Thus the metabolic processes influenced by diet are also important components in the etiology of dental caries. The major metabolic effects of diet upon tooth decay seem to operate prior to the time when the teeth have developed (Shaw, 1949).

Shaw (1950), applying the Sogannes (1948) prenatal and preweaning cariogenic feeding techniques, rendered his caries-resistant rats susceptible to tooth decay.

Bunting (1935) believed that the dietary factors influence dental caries through the determination of the environment of the teeth rather than through changes in the resistance of the tooth itself.

Hoppert, Webber and Canniff (1932) were successful in producing experimental dental caries in rats by the inclusion of coarse particles of grain in their diets. Less dental caries resulted when fine particles were used. Liberal amounts of vitamins A and D, as well as supplements of calcium and phosphorus did not appreciably retard the tooth decay resulting from the inclusion of coarse particles in the diet. These findings were confirmed by Rosebury and Karshan (1935) who prevented experimental caries by using rice ground to flour fineness. Sogannes (1948) believed that the experimental caries produced in this manner were due to mechanical breakages and subsequent

enlargement of the lesion produced. Van Huysen (1950), however, observed that while some minor fracturing was present on a diet containing large particles, the teeth did not fracture unless the cusps were first undermined by dental caries.

Nakfoor, Hunt, and Hoppert (1952) tested the Hunt-Hoppert rats for resistance to breakage of the cusps of the lower molar teeth. It was found that the caries-resistant rats showed a greater resistance to breakage than did the caries-susceptible rats. Fracturing, however, was not found to be an important factor in initiating dental caries in these rats.

These investigators were not able to duplicate the results of Braunschneider, Hunt and Hoppert (1948) in preventing dental caries by using flour-fine rice in the diets of the Hunt-Hoppert strains. In view of the fact that Nizel and Harris (1951) observed that foods grown on different soils had cariogenic properties, it may well be that these conflicting results were due to different origins of the foodstuffs. The rice used could have been nutritionally quite different though ground to flour fineness in both cases.

Kifer (1953) believes that the sulci of the molar teeth of the Hunt-Hoppert susceptible and resistant rats differ somewhat. The sulci in the molar teeth of the caries-susceptible rats are wider than those in the caries-resistant strain. These differences would presumably be important in the impaction of food within the sulci.

Clise and Hunt (1953) observed that the rates of growth of the susceptible and resistant strains were essentially the same for the first 21 weeks in males and 40 weeks in females, after which differences in growth appeared. At one year of age susceptible males and females weighed significantly less than the resistant males and females. The causes of this deviation are unknown.

The caries-producing diet used in the present study. The composition of this diet, by weight, was 66 percent ground polished rice, 30 percent whole milk powder, 3 percent of alfalfa leaf meal and 1 percent of sodium chloride. The rice used was passed through a precision grinder, adjusted so that about 2 or 3 percent of it would be retained on a 20-mesh screen when sifted. This ration is used in the colony of Hunt and Hoppert as a standard diet.

Dietary supplement of trace minerals. In order to determine whether or not a dietary supplement of vitamins and minerals would alter the response to dental caries in susceptible series rats, a commercial product "Vita-D-Mineral Supplement", manufactured by Vitamineral Products Company, Peoria, Illinois, was added to the standard caries-producing ration in a preliminary study (unpublished data).

The ingredients of this supplement were stated by the manufacturer to be as follows: ground limestone, defluorinated phosphate, dicalcium phosphate, steamed bone meal, vitamin D,

brewers yeast and trace minerals (Appendix VI). The supplement was added to the standard diet in the ratio of four parts per hundred and fed to two litters of susceptible series rats (pre- and postnatally).

The results were not conclusive but it was evident that this dietary supplement did have some effect on the incidence of dental caries in the Hunt-Hoppert rats. The rats themselves exhibited good growth and their incidence of dental caries appeared to be reduced. This study was not continued but the results are suggestive of a possible line of investigation for the future.

Experimental Procedure

The lower counts of lactobacilli in the mouths of caries-resistant rats observed by Jay, Hunt and Hoppert (1944) suggest that the salivas of the two strains are chemically different. If that is true, the elimination of the secretions from one or more of the salivary glands might, conceivably, cause the two strains of rats to develop caries in about the same length of time if the differences in the strains were due to salivary secretions. It is a simple procedure to sever the parotid duct, so this operation was carried out.

All animals used were produced according to the standard procedures of the project. The controls were from rats produced by Hunt and Hoppert in the prosecution of their experiment on the hereditary factors. After one or more litters of young had been produced for Hunt and Hoppert's work, certain of the

breeding females were used to raise young for the parotid gland experiment. Full sibs produced in earlier litters were used as controls. All animals were fed the standard diet of the colony.

When the experimental animals were thirty-five to forty days old they were anesthetized with ether, the hair covering the sides of the head removed and the exposed skin cleaned with alcohol. The rat was held on its back and an incision made in the skin of the head anterior and ventral to the ear on the side to be operated on. The parotid duct was located and a section of it removed. Care was taken not to injure the facial nerve which courses parallel to the parotid duct in this area (Greene, 1935). This was desirable since Jarbak (1950) showed that resection of this nerve in the rat caused paralysis in the facial muscles. This paralysis resulted in an increased incidence of dental caries, presumably due to a lack of buccinator function which resulted in increased deposits of food along the side of the third and second molar teeth.

A portion of the parotid gland was removed along with the duct. It was not considered advisable to remove all of the gland because its extirpation severs several fibers of the great auricular nerve derived from the cervical and brachial plexuses which emerge from the shoulder and penetrate the parotid (Cheyne, 1939a). The blood supply to the parotid gland is very rich. The parotid covers the posterior auricular artery which may be broken by operative procedures for the complete removal of the parotid gland.

The exposed area was sprinkled with veterinary uride powder to prevent infection and to promote healing. The incision was closed with wound clips. As soon as the surgical procedures were completed on one side, they were repeated on the other side. Each animal was then ear-notched for identification purposes and placed in a clean cage. When the wound had healed sufficiently the clip was removed. There was no case of serious infection.

All animals were kept in cages made of galvanized sheet steel closed on all sides except the front and top, which were covered with one quarter inch galvanized steel mesh. Cages measured twelve inches in height, fourteen inches in width and twenty inches in length. From one to five animals were kept in a cage at one time. The number of rats usually did not exceed four, however, and all were of the same sex. Wood shavings were used as litter. Water was available at all times from a drip bottle.

The animals were kept in a large, well lighted room maintained at a temperature of approximately 78° F. There were no means of preventing higher temperatures during the summer, when, occasionally, higher readings were noted.

The experimental and the control rats were inspected at bi-weekly intervals for the presence of macroscopic dental caries. This inspection was made with the unaided eye. The tongue was pushed aside and the jaws held apart by a nasal speculum. Light was supplied from an ordinary desk lamp. The

animal to be examined was held firmly by an assistant who grasped it by the loose skin at the back of the neck with one hand and prevented bodily movements with the other hand.

When macroscopic dental caries were observed for the first time, this fact and the animal's age at that observation were recorded. This age in days minus a constant factor of thirty-five days was called the "caries time" of a rat. At the outset of Hunt and Hoppert's experiment the ground rice in the ration contained about seventy percent of particles that would be retained on a 20-mesh screen. This diet was introduced when the rats were thirty-five days old. This ration mechanically injured the upper molars so it was exchanged for a diet, present from birth, which contained rice having two or three percent of the coarse rice particles. Since that exchange, thirty-five days have been subtracted from the age at which caries first appeared to make the earlier and the later data comparable.

All experimental rats were autopsied after they developed dental caries to verify the removal of the parotid duct.

Results. The removal of parotid secretion did not markedly alter the time of onset of the first carious lesion. The susceptible series rats developed caries twelve days later, on the average, than the susceptible controls. This difference, however, was less than the number of days between observation periods for carious cavities (Table 1). The "t" test (Snedecor, 1946) was used to test this difference for significance.

TABLE 1

CARIES TIME FOR PAROTIDECTOMIZED AND COLONY CONTROLS

Series	Susceptible		Resistant	
Treatment	Intact	Parotid-ectomized	Intact	Parotid-ectomized
No. of rats	85	55	160	60
Avg. caries time	39±2*	51±3*	479±15*	436±30*
Avg. periods	2.8±.96	3.5±.09	34±1	31±2
"t"	0.78		0.10	
No. of sibships	13	11	28	12
Avg. of sibship means	40±3	55±8	487±63	452±35

* ± Standard error of the mean.

Since t = 0.78, the difference was not significant and could be attributed to chance alone. The resistant series parotidectomized rats required forty-three days less, on the average, for the first appearance of dental caries than the colony controls. This difference is approximately three observation periods. The resistants were extremely variable so that this difference was not significant (t = 0.10).

The mean caries time within each sibship was also computed and these means were averaged for a value, the average of the sibship mean. These averages for both series of rats are also presented in table 1. Complete caries times for all rats are presented in Appendix I.

Conclusions. Cheyne (1939b) showed that parotidectomized rats had slightly more carious teeth on a diet containing coarse particles. Once initiated, caries were said to be more rapid in the rate of development in parotidectomized rats. Whether the onset of dental caries was altered, however, is not stated. If there was any increase in the rate of development of the carious lesion subsequent to its initiation, the method of observation in the present investigation would not bring it out.

It has been demonstrated that the inherited difference between the caries susceptible and caries resistant Hunt-Hoppert strains is not to any considerably degree due to the secretions of the parotid gland. The behavior of rats of the two strains remains essentially the same with respect to the development of dental caries when they are parotidectomized as when they are intact.

III. EXPERIMENTAL STUDIES OF THE THYROID GLAND

Recovery of Radioactive Iodine from the Thyroid Gland following the Intraperitoneal Injection of Tracer Quantities of I^{131}

Iodine has been demonstrated to be a very essential element in vertebrate metabolism. The relationships between the function of the thyroid gland in vertebrates and the metabolism of iodine have been the subject of numerous investigations, particularly since the advent of radioactive tracer techniques. Iodine has a number of radioactive isotopes. Currently I^{131}, which has a half life of eight days, is used almost exclusively. Iodine^{131} produces both gamma and beta radiation but the biological effect of the gamma rays is usually negligible because of the very small absorption of these rays by the thyroid (Chapman and Evans, 1946).

The administration of large quantities of the radioactive iodine will damage the thyroid gland by internal beta radiation. Since the parathyroid gland in the rat is largely within the thyroid gland, it too becomes damaged by the internal radiation (Gorbman, 1947). Sufficiently high doses of I^{131} will cause curtailment of growth, failure of functional and regenerative processes of the thyroid gland and parathyroid injury, as well as recurrent nerve injury, presumably due to the large quantities accumulated in the thyroid gland (Gorbman, 1950).

Winchester, Comar and Davis (1949) completely eliminated the thyroids of young chickens by I^{131} irradiation apparently

without damage to organs or tissues other than the thyroid and parathyroid glands. Replacement therapy with d,l-thyroxine in a water suspension allowed the birds to grow normally. On the other hand, however, with radioactive iodine in the quantities ordinarily used in tracer studies there is no apparent injury to the thyroid gland (Gorbman, 1950, and Pearlman, et al. 1941).

When iodine is administered to normal animals, it is accumulated and deposited in the thyroid gland. The rate at which this occurs is dependent upon the amount, the form and manner in which it is administered as well as the amount present in the animal's body and in its diet. In addition, it has been shown that the thyroid gland of the rat is dependent on the presence of thyrotropic hormone for its capacity to concentrate iodine from the serum, to bind iodine to protein and to discharge thyroid hormone (Vanderlaan and Greer, 1950). Administered doses of I^{131} enter the thyroid gland as inorganic iodine and this is rapidly transformed into diiodotyrosine and as such may be deposited in the gland (Leblond et al., 1946). The presence of radioactivity in many but not all follicles of the gland as early as one hour after injection shows that follicles differ in their ability to fix iodine actively. In general, the less active follicles are found under the capsule and in the isthmus of the gland (Leblond and Gross, 1948).

Experimental Study

A standardized tracer dose of carrier-free radioactive iodine (I^{131}) was injected intraperitoneally into a total of 184 rats of the two strains. Rats were sacrificed by etherization at intervals of 18, 68 and 148 hours after injection. The thyroid glands were immediately removed, cleaned of connective tissue and weighed on a Roller-Smith balance. The thyroids were then placed on small copper discs and allowed to dry, after which they were counted with a thin end window G.M. tube. At the time of administration, a measured amount of the injection solution was placed on similar discs and dried. These standard discs were counted at the same time as those containing the thyroid glands, thus eliminating the necessity of correcting arithmetically for the physical decay. The thyroid I^{131} present at the times of sacrifice are summarized in table 2 and appendix table II. The standard error accompanies each mean.

At 18 hours after injection an average of 4.65 percent of the injected dose was recovered in the thyroids of the susceptible series. In the resistant series the comparable recovery was 4.25 percent, which was not significantly different.

At 68 hours the percent of administered dose present was determined in four separate groups of animals from three experiments. Inspection of the values within strains shows that the groups were essentially similar and that they might then be grouped into a single larger category for comparison between strains. The thyroids of the resistant strain contained an

average of 3.55 percent of the injected dose. Those of the susceptible strain contained 5.57 percent of the injected dose. The difference between these means is highly significant.

At 148 hours the thyroid I^{131} activity was determined in three groups of rats of each strain. Since these three groups were also found to be essentially similar they too were grouped into larger categories for comparison between strains. The thyroids of the resistant strain contained an average of 2.69 percent of the injected dose. The comparable recovery was 4.58 percent from the susceptible strain. These means are significantly different. The mean values at each of the three times of sacrifice have been plotted in figure I.

The I^{131} retention values found in these rats are somewhat lower than those reported for the Sherman Strain of rats by Meites and Wolterink (1950) who obtained about 7.9 percent retention at eight hours. Cortell and Rawson (1944) report uptake values at four hours between four and thirteen percent, also with the Sherman Strain of rats.

Other strains of rats on other diets have considerably higher uptake values, however. Keating et al. (1945) determined I^{131} uptake to be about 17 percent, while Morton et al. (1942) found uptake values of I^{131} to be over 60 percent at four hours. Pearlman et al. (1941) found that up to 65 percent of an administered dose of I^{131} was taken up by the thyroid between 25 and 50 hours. McGinty (1949) and Jones (1951) showed that the amount of iodine retained by the thyroid gland

may vary with the degree of hyperplasia and with the dietary levels of iodide. Differences which have been reported from different strains of rats could be due in part to differences in either of these variables as well as to functional differences. In the present study all rats were fed ad lib. the standard caries diet of Hunt and Hoppert which contains 1.0 percent salt to which has been added 0.01 percent potassium iodide. Since this level is present, the thyroid glands of the experimental animals were saturated with dietary iodide. Thus there was a smaller percentage uptake of the injected dose of radioactive iodine which may be attributed to this fact.

The differences in the uptake-retention curves of the susceptible and resistant strains then represent a faster turnover rate of I^{131} in the resistant strain. From the data of this investigation, it appears that the turnover rate of the resistant strain is about 42 percent greater than that of the susceptible strain.

TABLE 2

AVERAGE VALUES OF PERCENT RECOVERY OF I^{131}

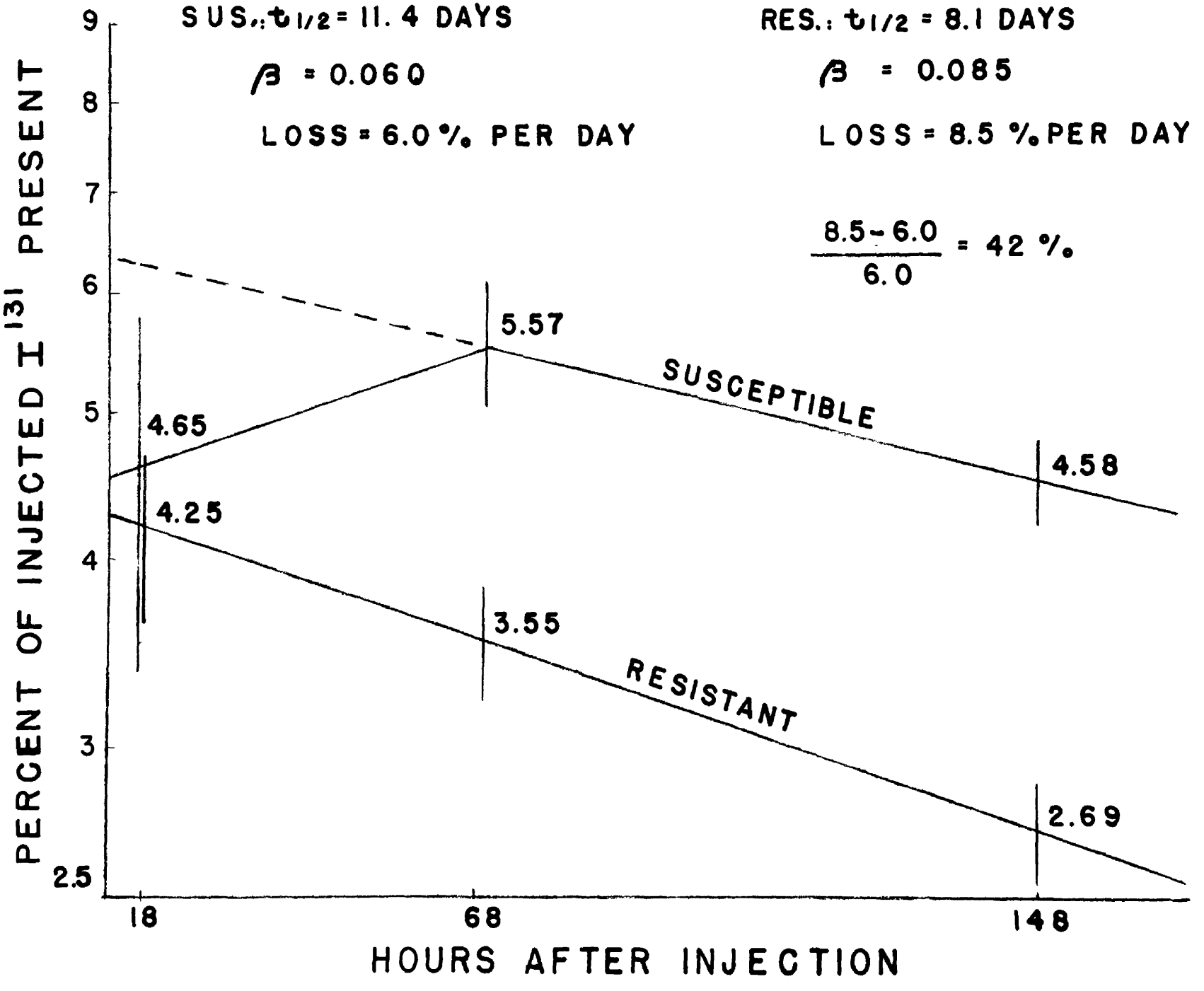
Time (Hours)	Resistant		Susceptible	
	I^{131} sem*	No. of rats	I^{131} sem*	No. of rats
18	4.25±0.28	9	4.65±0.62	5
68	3.55±0.16	39	5.57±0.25	50
148	2.69±0.11	32	4.58±0.15	49

* Standard error of mean

FIGURE I

Retention of Radioactive Iodide
by the Thyroid Gland

Mean values, plus and minus twice the standard error, are plotted. The retention curve is drawn from the mean values.


SUS.: $t_{1/2}$ = 11.4 DAYS
β = 0.060
LOSS = 6.0 % PER DAY
RES.: $t_{1/2}$ = 8.1 DAYS
β = 0.085
LOSS = 8.5 % PER DAY
$\frac{8.5-6.0}{6.0}$ = 42 %
PERCENT OF INJECTED I^{131} PRESENT
9
8
7
6
5
4
3
2.5
5.57
4.65
4.25
4.58
3.55
2.69
SUSCEPTIBLE
RESISTANT
18
68
148
HOURS AFTER INJECTION

Weight of the Thyroid and Pituitary Glands

Weight of the Thyroid Glands. When differences in the gross thyroid weight of a number of rats of the two strains were first noted, it was considered advisable to treat all available data statistically to determine whether or not these variations were the result of chance observations or whether true differences did exist.

Among normal rats under standard environmental conditions the thyroid gland is nearly constant in size. Thyroxine is produced in response to the stimulus of the thyrotropic hormone of the anterior pituitary gland. When the thyroxine level drops below the required levels for an individual animal, the thyrotropic hormone of the anterior pituitary causes enlargement of the gland and increased production of thyroxine. Conversely, when a thyroid gland produces an excess amount of thyroxine, this excess causes a reduced amount of thyrotropic hormone to be produced, which in turn causes a reduction in the production of thyroxine by the thyroid gland. A fine reciprocal control is thus exhibited by the thyrotropic hormone of the anterior pituitary gland and the thyroxine of the thyroid gland. This control is responsible for the constancy of thyroid gland weights usually observed among experimental animals.

Hyperemia and enlargement of the thyroid gland may be produced under experimental conditions as is stated below, but as previously mentioned the gland-body weight relationships are considered to remain fairly constant under normal conditions.

The thyroid glands of all rats of the investigations concerning I^{131} uptake and the assay of the rate of secretion of the thyroid hormone, were dissected from the animals. The glands were weighed to the nearest 0.01 milligram on a Roller-Smith precision balance. The body weight was taken to the nearest gram. All gland weights were expressed as gland weight in milligrams per one hundred grams body weight.

The difference in thyroid weight per 100 gms. body weight among the various groups was examined statistically by means of the "t" test (Snedecor, 1946).

The difference between the males and females of each strain was found to be non-significant (susceptible series $t = 1.12$, resistant series $t = .50$, Appendix III). On this basis, then, the males and females of each strain were combined for a comparison between the strains. Table 3 shows that the average weight of the thyroid gland expressed as milligrams per one hundred grams body weight in the susceptible series was 8.03 ± 0.13 and the corresponding value for the resistant series was 5.62 ± 0.15. The difference of 2.41 is highly significant, ($t = 12.2$). The thyroid glands of the susceptible series rats are about 43 percent larger than are those of the resistant series rats, when they are compared in this manner.

TABLE 3

AVERAGE WEIGHTS OF THE THYROID GLAND

Series	No. of rats	Mg. / 100 Gm. B.W.	Standard Deviation
Susceptible	118	8.03±0.13*	1.42
Resistant	114	5.62±0.15*	1.58
Total	232		
t = 12.2, highly significant			

* Standard error of the mean.

Weight of the Pituitary Glands. The pituitary glands were removed from twenty-two susceptible series rats and seventeen resistant series rats. These glands were weighed on a Roller-Smith torsion scale and the weights compared on a gland weight basis as well as a gland weight per one hundred grams body weight basis. The results are summarized in table 4.

The average gland weight from susceptible animals was 6.0±0.41 and that of resistant animals, 5.0±0.19 milligrams. The difference of 1.0 mg. between the two values is not statistically significant ("t" = 0.6).

The average gland weight expressed as milligrams per 100 grams body weight, for the susceptible series animals, was 3.4±0.34 and for the resistant series was 3.9±0.17. The difference between these two values of 0.5 is again non-significant ("t" = 0.3).

Thus there was no apparent difference in gross weights of the pituitary glands between the two strains of rats.

TABLE 4

AVERAGE WEIGHT OF THE PITUITARY GLANDS

Strain	Number	Wt. in milligrams	Wt. in mg. per 100 gm. B.W.
Susceptible	22	6.0±0.41*	3.4±0.34*
Resistant	17	5.0±0.19*	3.9±0.17*
"t" value		0.6	0.3

* Standard error of the mean.

Respiratory Metabolism

When apparent differences in iodine metabolism and gross size differences in the thyroid gland were noted, it was considered advisable to determine whether or not there were differences in gross energy metabolism between the two strains of rats. Metabolism will vary according to a large number of conditions. Among these may be mentioned season, activity, and temperature variants.

Seasonal rhythms of one sort or another are present in most animals. It is known that thyroid activity is sensitive to changes in temperature and light irradiation, and this factor along with anterior pituitary and gonadal factors may be at least partly responsible for some variations. Sherwood (1936) observed that normal adult rats exhibited up to 26 percent diminution in metabolism during summer months. The larger animals showed the decrease to a lesser extent than did smaller animals. Benedict and MacLeod (1929) observed that heat production as measured in gaseous metabolic studies is lower in the summer, and their values ranged to a 12 percent decline.

It is well known that an animal working, or at exercise, consumes more oxygen than does an animal at rest. For this reason studies of basal metabolic levels must be made with the animal at rest. Benedict and MacLeod (1929) found that very mild activity in rats did not alter oxygen consumption values, and they concluded that ocular observations are sufficient to estimate activity. Observations on animals which are excessively active would not be considered as basal but would be

considered as representing values obtained under conditions of exercise.

The influence of age and size of animals are of importance in such determinations. Brody and Procter (1932) measured metabolic level and size in a large series of animals and found that basal energy varies as the (0.73) power of body weight $\left[(\text{Body weight})^{.73}\right]$. They suggested that this correction be used in making comparisons among animals of different body weights. Belasco and Murlin (1941) proved that the basal metabolism of normal rats is higher in young animals than in adults. The level declines rapidly during the first few months, then becomes rather constant.

As mentioned in the discussion of thyroid secretion rates, there may be some differences between the sexes in this respect. Several investigations, however, indicate little or no differences in gaseous metabolism. Davis and Hastings (1934) failed to find any differences in rats up to four months of age in a series of 136 animals. Barker (1945), likewise, found no differences between the sexes. On the other hand, Benedict and MacLeod (1929) observed that male rats have a higher metabolism until an age of 14 months.

Soliman (1952) observed that female rats in estrus consumed more oxygen than did these rats in other phases of the estrus cycle. Lee (1928), however, in a similar study found that there was a significant increase in metabolism of rats during the last ten hours of diestrus and the first six hours of proestrus. There was no change in metabolic level in the

other phases of the estrus cycle. Some of the variation observed in female rats would seem to be due to the fact that there is some variation in metabolic rates during the several phases of the estrus cycle.

The nature of the food which an animal has been eating has some effect on gaseous metabolism. Horst, Mendel and Benedict (1934) studied this influence. Many types of balanced rations in rats gave essentially uniform results. A diminished protein intake prior to the determination, however, generally caused a reduced metabolism in the animal. These investigators studied the effects of prolonged fasting and found the oxygen consumption dropped steadily and rapidly until the animal died. Benedict and MacLeod (1929) observed that the metabolism of the rat is depressed ten percent by twenty-four hours fasting. They suggest that in rats a seventeen hour period of fasting prior to gaseous metabolism checks is adequate to eliminate the complications of diet, so that the animal is at a basal level.

Cori and Cori (1934) showed that the metabolism of a fasting rat is 90 percent fat oxidation, and hence because of this fact as well as the difficulty in making urinary collections, it is not considered necessary to make corrections for the nitrogen content of urine in determinations involving fasted rats.

Horst, Mendel and Benedict (1934) observed that the rat is subject to diurnal variation. It was observed by these workers that oxygen consumption in the rat is high early in

the morning and in the late afternoon. Because of this variation, it is suggested by them that all determinations be made after 10 o'clock in the morning and before 4 o'clock in the afternoon. In addition to this, it has been suggested elsewhere (Benedict and MacLeod, 1929) that determinations involving rats be made in a well-lighted room and that the unit chamber be lighted so that the rat will be quieter.

Since the rat is a homeothermic animal, any gross changes in environmental temperature will necessarily involve internal metabolic changes which counteract these external changes. The temperatures at which metabolic studies are made influence the net results. Using several environmental temperatures, Benedict and MacLeod (1929) found that 28° C. was the optimal temperature. Values obtained below 28° C. were not found to be as valid as those at 28° C. or slightly above. Rats maintained in an environment of 16° C. may consume up to twice as much oxygen as rats at 28° C. (Horst, Mendel, and Benedict, 1930).

Certain exogenous substances have a marked influence on bodily metabolism. Since the work of MacKenzie and MacKenzie (1943) and others, goitrogens have been used widely to lower metabolic levels. It was found that the basal metabolic rate could be lowered as much as twenty percent in ten to fourteen days. Meyer and Ransom (1945) reported values as much as forty percent below normal by the use of goitrogens.

Potent goitrogens have the same metabolic effect as does thyroidectomy in that the animal is deprived of the hormone

thyroxine and the metabolic level drops (Astwood et al., 1943). Rats receiving thiouracil show a slower decline in metabolic rate and heart rate than do thyroidectomized rats but they eventually reach the same level (Meyer and Ransom, 1945). Presumably this is because thiouracil inhibits the production of new thyroxine but it does not interfere with the use of any which is stored and thus is available (Halpert, Cavanaugh and Keltz, 1946).

As an experimental tool in lowering the metabolism, a potent antithyroid drug has some advantages over thyroidectomy insofar as the parathyroids are not damaged and any scattered thyroid tissue is reached by the drug.

When exogenous thyroxine is supplied to thyroidectomized animals or to thiouracil-treated animals the metabolism may be restored to nearly any desired level. Individual variations in thiouracil-treated rats are considerably larger than in thyroidectomized animals, and their response to ingested or injected standard doses of thyroid substances are somewhat erratic and irregular (Meyer and Ransom, 1945).

The administration of exogenous thyroxine to normal animals does not necessarily raise their metabolic level. It may simply set in motion mechanisms which serve to suppress the bodily production of thyroxine. To raise the metabolic level the administered thyroxine must be in excess of that which the animal would normally produce.

Meyer and Wertz (1939) increased oxygen consumption 25 to 30 percent with the administration of thyroxine. In addition to raising metabolic levels as evidenced by increased oxygen consumption, thyroxine administration also causes body weight losses in mature animals or reduced rates of gain in growing animals (Belasco and Murlin, 1941). Reisfield (1950) found that exogenous thyroidally active substances caused decreased resistance to anoxia as evidenced by survival time in closed jars. This was because metabolic levels were raised and oxygen requirements were increased.

Greenberg (1952) found that vitamin B_{12} has no appreciable effect in counteracting the growth retarding action of dietary desiccated thyroid powder in rats while methyl linoleate plus cottonseed oil administration was found to protect the rat against such retardation.

The health and general condition of animals in metabolic studies are important inasmuch as poor health may seriously alter such levels and serve to confuse the investigator or even invalidate his results. Physiologically abnormal rats show extreme variations in metabolic levels (Benedict and MacLeod, 1929).

The measurement of oxygen consumption in normal rats. The oxygen consumption of male rats of both strains was checked in closed circuit metabolism units after the method of MacLagan and Sheahan (1950). The rats were placed in calibrated desiccators filled with oxygen and which contained a pan of soda

lime. The air was partially removed from the desiccator and system with a vacuum pump and oxygen was added from a Douglas bag. This system was connected to a U tube filled with mercury. Pressure readings were made at fifteen-minute intervals for a period of an hour and a half or more. The carbon dioxide produced by the respiring rats was absorbed by the soda lime. The amount of oxygen used by the rat then was determined by converting the pressure changes in the system by the use of the following formula:

$$(V_d - V_r) \times \frac{P}{760} \times \frac{273}{T} \times \left(\frac{\text{Rat Wt.}}{100} \right)^{.73} = O_2$$

Where V_d = Volume of desiccator

V_r = Volume of the rat assuming 1 gm. = 1 cc. Vol.

P = Fall in pressure in mmHg per hour

T = Absolute temperature inside the desiccator

O_2 = Oxygen consumption per unit of body weight adjusted to the .73 power

The rat weight was adjusted by using the .73 power because the range in body weight was too great to use a simple correction. Body weights ranged from 195 to 408 gms. Brody and Procter (1932) suggest using this correction when comparing animals of different body weights because they find that the metabolism of mammals varies with the .73 power of body weight.

The determinations were made in an air conditioned room maintained at a constant temperature ($24 \pm 1^{\circ}$ C.) and constant

humidity. Prior to the test the rats were maintained on the standard caries-producing diet of Hunt and Hoppert. Food was withheld for at least 17 hours preceding the tests, but water was avilable except during the actual oxygen determination. The units were arranged in a bank of twelve. Thus it was possible to run determinations on six rats of each strain at one time. The purpose was to compare the two strains with each other.

The rat was placed in the desiccator and the unit sealed. Air was evacuated by means of a vacuum pump which produced a pressure change of about 150 mm. Hg. and oxygen was added from a Douglas bag. The unit was allowed to reach an equilibrium temperature during which time the rat also became quiescent. This preliminary period lasted at least one-half hour after which the pressure inside the unit was adjusted to equal the atmospheric pressure by adding oxygen. After an additional period of five minutes the readings were begun on a fifteen minute schedule for at least six uniform readings.

Oxygen consumption measurements were not begun until the animal had been in the chamber for one half hour in order to (1) accustom the animal to the chamber, (2) bring the system to equilibrium temperature, (3) establish an equilibrium between the absorbing rate of the soda lime and the carbon dioxide production rates. The temperature within each unit was $26 \pm 0.5^{\circ}$ C.

Table 5 shows the average oxygen consumption for the several groups of rats of each strain. Oxygen consumption is expressed both as cc. used per 100 grams of body weight of the animal and as cc. per 100 gm.$^{.73}$. Data on the age and body weights of these rats is given in appendix IV. The consumption of oxygen of the two strains was compared by the use of the method of analysis of variance of the original data. The results of such computations are shown in appendix IV. The caries-susceptible rats used slightly more oxygen per unit of time than did the caries resistants. Because of the variations present, however, this greater amount is not statistically significant. This is the case whether the oxygen consumption is determined on a 100 gm. body weight basis or adjusted to the .73 power of body weight.

The individual chambers used in the oxygen consumption determinations were not always used for the same strain of rats. The strains were alternated after each run, thus it was possible to compare chambers in the various runs to determine whether or not these chambers were concealing any biological variation. The comparison was made using the method of analysis of variance (appendix IV). The values F for chambers of 0.75 and F for runs of 1.41 indicate that there were no significant differences in the individual chambers or in the successive runs which would conceal biological variation if any were present in sufficient quantity to be measured with the method employed here.

TABLE 5

OXYGEN CONSUMPTION OF UNTREATED RATS OF BOTH STRAINS

Strain	Run	cc. per $\left(\frac{B.W.}{100}\right)^{.73}$	cc. per 100 gm. B.W.
Susceptible	1	124.82±3.99*	89.2±3.24*
Susceptible	2	136.94±6.96	100.7±4.81
Susceptible	3	146.14±4.81	113.8±5.38
Susceptible Av.		135.96±3.70	101.2±3.47
Resistant	1	128.45±4.20	89.8±3.27
Resistant	2	135.08±4.90	96.0±4.06
Resistant	3	121.17±3.26	94.0±2.75
Resistant Av.		128.12±2.74	93.2±1.94

* Standard error of the mean.

Induced hyper- and hypothyroidism. Hyperthyroidism was induced by oral administration of protamone, an iodinated casein product having thyroxine activity. This thyroactive iodinated protein is effective when given orally (Reineke, 1949). The lot of protamone used in this work was assayed by means of the isotope dilution method. These assays indicate that the thyroxine activity was probably about 1.0 to 1.2 percent (Reineke, 1953).

Hypothyroidism was induced by using thiouracil administered as 0.2% of the standard caries producing diet. Protamone was

administered in three dosage levels. These were 0.02, 0.05 and 0.1 percent of the standard caries producing diet. Controls were maintained on the standard diet alone.

All the rats used in the experiment were produced by brother-sister mating of animals of the caries-susceptible strain of Hunt and Hoppert. Up until the time of weaning, the animals were kept with the parental female and the standard caries producing diet was available. At 25 days of age the young were separated from their mother and fed the modified diets as indicated. Each rat was weighed once a week and it was examined for the presence of dental caries, according to the method described above. As soon as at least one carious lesion appeared in any lower molar tooth, the rat was considered as showing dental caries. (The age of the rat when caries first appeared in any of its lower molars minus a factor of 35 days is considered as the rat's caries time.)

Protamone in the quantities given caused the rats to show a reduced rate of growth and a higher rate of metabolism than the controls. The level of 0.1 percent proved to be above the tolerance dose in these rats and caused death. The rats treated with thiouracil were 30 to 60 percent below normal in weight.

There was no great effect on caries time in the rats, although rats on 0.02 percent protamone did show a somewhat increased resistance. Table 6 shows the dosage levels, number of rats, sex, and average caries times for the various groups.

These data were treated using the method of analysis of variance with corrections for disproportionate sub-class numbers (Snedecor, 1946). The completed analysis of variance gave the results shown in appendix IV. The caries times were not significantly altered by the treatments of hypo- and hyperthyroidism.

TABLE 6

HYPER- AND HYPOTHYROIDISM AND DENTAL CARIES

Treatment	Females		Males	
	Rats	Caries time	Rats	Caries time
Control	4	24.0	4	21.7
0.2% thiouracil	6	20.8	4	27.3
0.02% protamone	5	48.0	5	34.2
0.05% protamone	6	25.7	4	15.4

F sexes = 1.25

F treatments = 0.11

F interaction = 0.99

This analysis shows that in the numbers used here the treatments had no significant effect on caries time of the susceptible series rats. It also shows that there was no difference in reaction of the two sexes to the treatments, nor was there any significant interaction between sexes and treatments.

The oxygen consumptions of a few of rats in each group were measured in a closed circuit type of metabolism unit. The unit used was modified after the Regnault-Reiset (1890) method by Reineke (1953). In this unit the rat was placed in a closed chamber with a water seal. As oxygen was used by the respiring rat, it was replaced from a graduated cylinder so that the quantity used could be measured. The carbon dioxide produced was removed from the system by a saturated barium hydroxide $\left[Ba\ (OH)_2\right]$ solution. Gases from the rat chamber were removed, passed over the alkali and then returned to the chamber by means of a rocking mechanism.

All rats were fasted prior to making the determination of oxygen consumption. The unit used had four chambers so four rats could be run at each trial. Readings of oxygen consumption were made at intervals of fifteen minutes over a period of at least two hours, and all volumes were corrected to standard conditions of temperature and pressure. Because all of the rats were fairly uniform in body size, the computations of oxygen consumption were made on a basis of one hundred grams body weight in each case. The results of this check are shown in table 7.

TABLE 7

OXYGEN CONSUMPTION OF RATS WITH INDUCED HYPER- AND HYPOTHYROIDISM

Treatment	No. of rats	O_2 per $\frac{B.W.}{100}$
Control	9	206.4
0.2 thiouracil	9	184.6
0.02% protamone	3	236.0
0.05% protamone	3	372.5

The numbers of rats used in this check were very small. The purpose was to determine whether or not protamone in the diet raised oxygen consumption and whether or not thiouracil lowered oxygen consumption. The data were not examined statistically but is it obvious that, on the average, thiouracil-fed rats used less oxygen than normal controls or the protamone-fed rats. The protamone-fed rats used more oxygen than did the normal rats or the thiouracil-fed rats. Within the protamone-fed rats, the rats receiving the higher percentage of this thyroactive substance required the greater amount of oxygen.

At the time the oxygen determinations were made the rats weighed 60-75 grams. For this reason these values are higher than those reported above for more mature animals of the strain since young growing rats have a higher consumption of oxygen than do adults.

Protamone fed to breeding rats. Several breeding females of the susceptible series rats (20th generation) were fed the standard diet to which had been added protamone.

Protamone was added as 0.025 and 0.013 percent of the diet and several females were kept with one male litter mate according to standard practice in this laboratory for the production of rats. One litter was born to a female on the 0.025 percent protamone treatment but the young (three) all died three days later. These were the only young known to have been born to any of the eight females in this series of trials. There were no young known to have been born to any of the eight females on the 0.013 percent protamone diet. There was a high mortality of both males and females and these trials were abandoned after sixty days. It was not considered possible to raise young on these dietary levels of protamone.

Thiouracil fed to breeding rats. In order to determine what effect dietary thiouracil would have on the incidence of dental caries in the molar teeth of growing resistant series rats, several were raised on a standard caries-producing diet to which had been added 0.2 percent thiouracil.

It has been demonstrated by Jones et al. (1946) that it is possible to raise young on a diet containing thiouracil provided the female parents were not on this diet too long prior to the onset of pregnancy. Complete sterility was not found but there was an altered estrus and a high incidence of resorption of embryos present.

Barker (1949) found also that it was possible to raise young from adult female rats under thiouracil treatment, but in this case the females were mated and fertilized while they were on a standard non-goitrogenic diet, and then they were switched to the goitrogenic diet after the onset of pregnancy. Thiouracil was continued in the diet of the female and then in the diet of her litter. It was found possible to raise cretinoid young until they were sixteen months of age, but their metabolism was reduced markedly and they showed lowered growth curves.

In the present study three resistant females were placed on a diet of the standard caries-producing ration containing 0.2 percent thiouracil seven days after mating with a litter-mate male. The male was left with the females in the breeding cage. The females were observed every four days for signs of pregnancy. Twenty-one days after the thiouracil was added to the diet one female died, at which time she was obviously pregnant. An autopsy showed that there were nine large fetuses present in the uterus. It appeared that it was impossible for birth to occur. Sixteen days later a second female died and an autopsy showed that young were also present in her uterus.

The third female and the male were continued on a diet containing 0.2 percent thiouracil for sixty days without the female ever showing signs of pregnancy, and then regular feed was substituted. Approximately sixty days later, this female produced and raised a normal litter. She was returned to the cage with the male and examined periodically for evidence of

pregnancy. The next time she showed signs of pregnancy she was placed on the diet containing thiouracil and a litter of five young was born five days later. These young were raised and weaned by the female in a nearly normal fashion. The five young all showed reduced vigor and lowered growth curves. They were observed periodically for dental caries. All five young died at an age of seventy days and their molar teeth did not show visible signs of carious lesions.

It appeared then that making resistant rats hypothyroid with thiouracil did not grossly alter the incidence of dental caries. For this reason no further attempts were made to raise rats with the thiouracil treatment.

Secretion Rate Assay

Active colloid appears in the thyroid gland of the rat after about 16 days of gestation, but 18 days marks the threshold of thyroid activity (Hall and Kaan, 1942). From this time on the rat thyroid secretes thyroxine which is necessary for its well-being and which affects nearly all tissues and organs of the body. The presence of thyroxine in the body of the rat is essential to growth, reproduction, and normal homeostasis (Jones, Delfs and Foote, 1946, and many others).

Biological assay of the thyroid secretion rate. The rate of secretion of thyroid substance by the thyroid gland has been the subject of many investigations and many methods have been suggested for this determination. The earlier methods were based on the observation of changes in the thyroid gland which

accompanied induced changes in its activity. The mitotic behavior of thyroid cells and the ocular measurement of thyroid follicles or of follicular epithelium were taken as indexes of thyroidal activity. Such indexes are useful for measuring the response of the thyroid gland to stimuli such as temperature shocks and the presence of thyrotropic hormone but they are not satisfactory for the determination of the actual rate of thyroid secretion. They are also time consuming and difficult to standardize.

The methods most frequently employed for estimating the rate of thyroid secretion have been based on the replacement of circulating thyroid hormone by crystalline thyroxine. The amount of exogenous thyroidally active substance or of crystalline thyroxine which is required to maintain thyroidectomized animals in a normal state is taken as representing the normal level of thyroidal secretion of that animal. The thyroidal substance is usually standardized on a basis of crystalline thyroxine. Thyroxine as produced in the laboratory consists of the racemic mixture of optically active dextro- and levo- forms. Biological assays of this racemic mixture and of the l-form show that l-thyroxine uniformly has about twice the potency of the mixture, hence it is concluded that the activity of the mixture may be accounted for by the l-component (Reineke and Turner, 1945). It has also been shown that the thyroxine present in the thyroid is the levo-rotatory form (Harington and Salter, 1930). Thus, when biological assays of thyroidal

material or of secretion rates are determined, these facts must be considered.

In the assay based on replacement therapy, the best criteria of normalcy are basal metabolic rate and heart rate. The growth rate of rats has also been used but this is an extremely laborious procedure and subject to errors.

Subsequent to the work of MacKenzie and MacKenzie (1943) most assays of thyroidal substances and of thyroid secretion rates have been done following the method of Dempsey and Astwood (1943). Test animals are made effectively athyroid by the use of a potent goitrogenic drug and graded doses of thyroxine or thyroidally active material are given simultaneously for a suitable period. The minimum amount of administered thyroxine which maintains a normal thyroid weight balance in the presence of the pituitary gland is considered the normal secretion rate in thyroxine equivalents. In the presence of the goitrogen, if too little exogenous thyroxine is supplied, the thyroid weight is greater than normal. Increasing the dosage of thyroxine reduces the gland weight. It has been demonstrated that this decrease in thyroid weight with increasing dosage of thyroxine compares closely with the increase in metabolic rate which also occurs. This was shown by Reineke, Mixner and Turner (1945), who concluded on this basis that thyroidal assays or measurements of thyroidal function by the goitrogenic technique were directly comparable with results obtained by the older and more laborious standard metabolic method.

Taurog and Chaikoff (1947) studied the rate of turnover of I^{131} in the thyroid gland with the method proposed by Zilversmit et al. (1943). The turnover rate times the iodine content is assumed to be equal to the thyroid output. This would not distinguish between the output of hormonal and non-hormonal iodine, however.

Several investigators have used the method of Dempsey and Astwood (1943) to determine the thyroid secretion rates in thyroxine equivalents. Purves (1943) using this technique and rape seed as the goitrogen found that 2.0 to 3.0 micrograms of thyroxine per hundred grams body weight would maintain the thyroids of male rats at a normal size. Reineke, Mixner and Turner (1945) found that approximately 4.8 micrograms of d, l-thyroxine were required to return thyroid weights of 140-gram rats to normal. Dempsey and Astwood (1943) found that 5.2 micrograms of l-thyroxine were required to maintain the thyroids at a normal level, but as pointed out elsewhere (Reineke and Turner, 1945), this l-thyroxine may have also had d-thyroxine present, which would account for the rather large value obtained.

Monroe and Turner (1946) investigated the thyroid secretion rate of rats during several phases of activity. These investigators found that the thyroid secretion rate in growing female rats ranged from 4.63 micrograms of d, l-thyroxine per hundred grams body weight for 50 to 99 grams body weight rats to 2.82 micrograms per hundred grams body weight for 250 to 300 gram body weight rats.

Griesbach and Purves (1943) employed subtotally thyroidectomized rats to estimate the normal rate of thyroxine secreted and found that a daily supply of 2.25 micrograms d, l-thyroxine per hundred grams body weight prevented hypertrophy or atrophy of the remaining thyroid fragment. They than assumed that this quantity (2.25 micrograms per hundred grams body weight) may be taken as equivalent to the normal secretion rate of the hormone in rats.

Monroe and Turner (1946) conclude that the normal secretion rate of thyroidal substance by the thyroid gland of the rat appears to be equivalent to two to five micrograms of d, l-thyroxine per hundred grams body weight per day.

Experimental results. Using the method of Dempsey and Astwood (1943), the rate of secretion of the thyroid gland expressed in equivalent amounts of crystalline d, l-thyroxine was determined for rats of the strain resistant to dental caries. The rats were kept in an air conditioned room maintained at 24±1° C. and fed the standard caries-producing diet described above. Thiouracil was mixed in the feed (0.2 percent by weight), which was fed ad lib. Rats were separated by sex and divided into five lots for each sex and graded doses of thyroxine were administered daily by intraperitoneal injections of a water suspension of the sodium salt. One group of each sex on the standard diet with thiouracil added received no thyroxine. In addition to these groups, a number of rats of each sex were maintained as a control with neither thiouracil in the diet nor

exogenous thyroxine being supplied. The treatment continued for two weeks, after which all animals were sacrificed. Animals were killed with ether anaesthesia, and the body weight of each animal was taken along with its respective thyroid weight. From these data the average thyroid weight per hundred grams body weight was calculated for each of the lots receiving the respective doses of exogenous thyroxine. These weights were then compared graphically with the values for the normal controls. The point of intersection of the curves of thiouracil-thyroxine-treated animals and the control weight line is taken as representing the estimated secretion rate of the rats expressed in equivalent amounts of crystalline d, l-thyroxine per hundred grams of body weight.

These data are summarized in table 8.

The estimated secretion rate of male rats of the resistant strain in this study is 1.95 to 2.02 micrograms d, l-thyroxine per 100 grams body weight. The estimated secretion rate of female rats is 2.1 to 2.2 micrograms per hundred grams body weight. In figures II and III thyroid weights are plotted using the average weight and showing plus and minus one standard error from this mean to indicate the levels of confidence provided by these data.

The secretion rates of the resistant series rats indicated by these assays are lower than values obtained by other workers using other rats. They are in agreement with those obtained by Griesbach and Purves (1943), however, who obtained values

indicating that the quantity 2.25 micrograms d, l-thyroxine per 100 grams body weight was equivalent to the normal rate of secretion.

Sufficient numbers of rats of the caries-susceptible strain were not available for a similar determination of their thyroid secretion rate.

TABLE 8

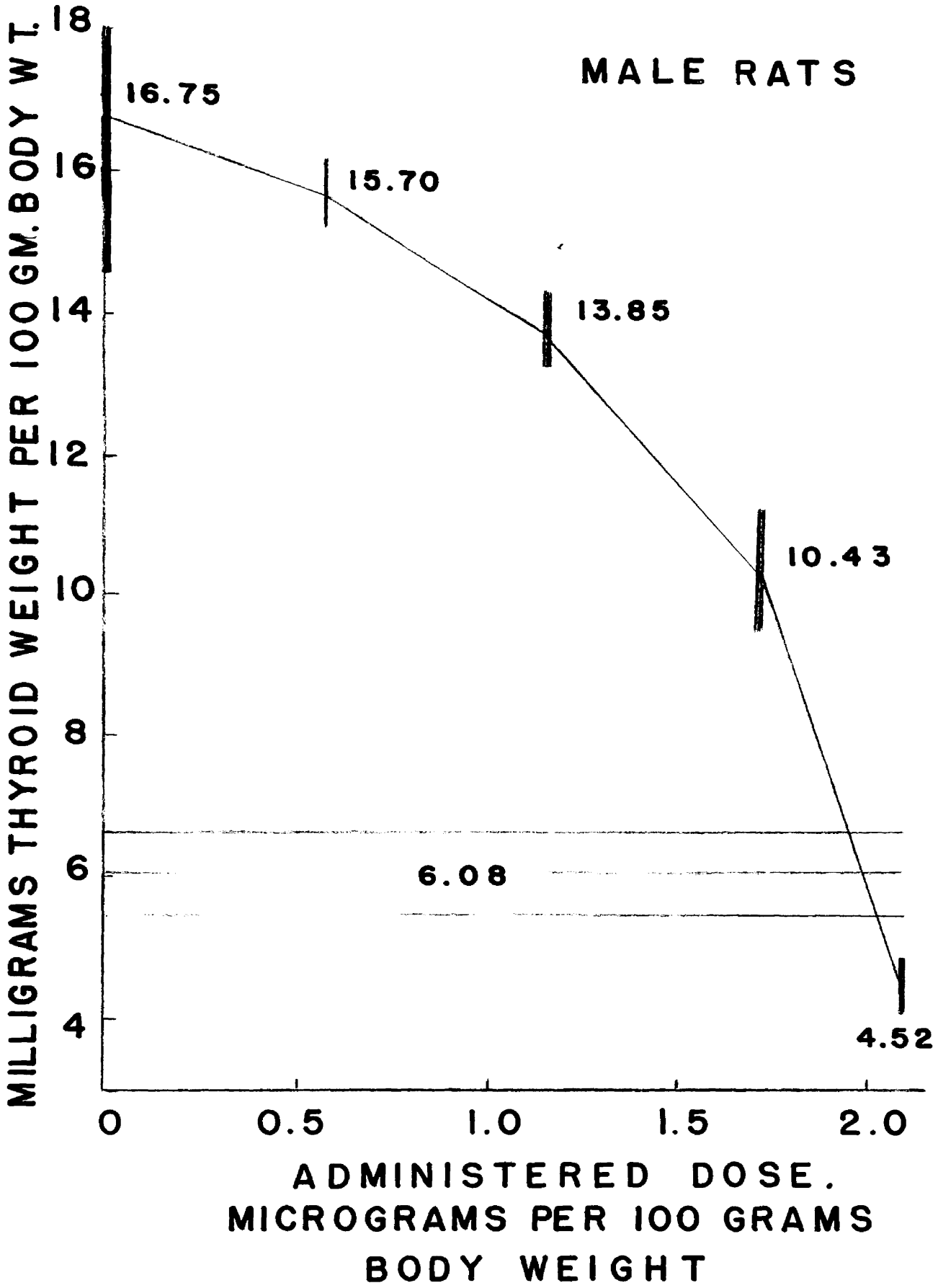
RATS' THYROID SECRETION RATE ASSAY DATA

Thiouracil in feed, %	No. of rats	Thyroxine µg. /100 g. B.W.	Avg. Body Weight	Thyroid Wt. µg. /100 g. B.W.
Males				
0	10	0	163	6.08±0.50*
0.2	5	0	196	16.75±2.22
0.2	5	0.57	176	15.70±0.43
0.2	5	1.15	173	13.85±0.56
0.2	5	1.71	175	10.43±0.91
0.2	4	2.09	191	4.52±0.33
Females				
0	13	0	132	5.35±0.58
0.2	5	0	150	22.73±1.15
0.2	6	0.76	139	22.90±0.90
0.2	7	1.44	131	15.30±1.13
0.2	6	2.10	143	5.58±1.51
0.2	7	3.01	133	5.04±0.82

* Standard error of mean.

FIGURE II

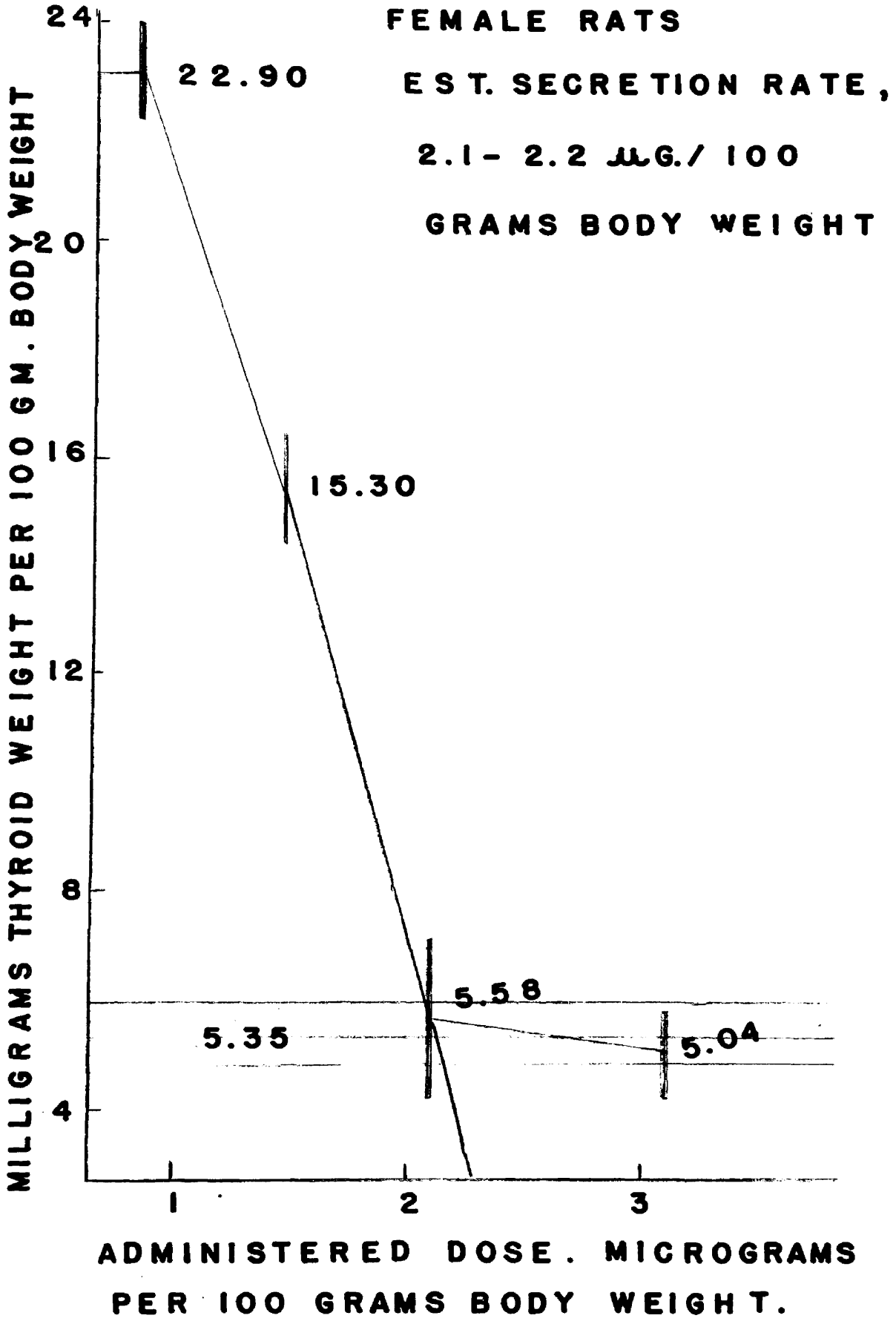
Method of Plotting Data to Determine Thyroid Secretion Rate of Resistant Series Male Rats



EST. SECRETION RATE, 1.95-2.02 mg./100 GRAMS BODY WEIGHT

FIGURE III

Method of Plotting Data to Determine Thyroid Secretion Rate of Resistant Series Female Rats


FEMALE RATS
EST. SECRETION RATE,
2.1 - 2.2 µG./ 100
GRAMS BODY WEIGHT
22.90
15.30
5.58
5.35
5.04
24
20
16
12
8
4
1
2
3
MILLIGRAMS THYROID WEIGHT PER 100 GM. BODY WEIGHT
ADMINISTERED DOSE. MICROGRAMS
PER 100 GRAMS BODY WEIGHT.

Histological Examination of Thyroid Epithelium

When differences were observed in the gross weight of the thyroid glands, it was considered advisable to examine histological preparations of these glands.

The thyroid gland is composed of lobules within which are the follicles. The follicles are the structural units of the thyroid gland. When seen in histological sections, the follicles are nearly always circular, but they may appear very irregular. The wall of the follicle is a layer of epithelial cells which vary from nearly flat to high columnar. There is no basement membrane and the cells rest directly on reticular tissue. There have been two types of epithelial cells described, "chief" cells and "colloid" cells, but it is thought that both of these merely represent different phases of secretory activities of the same type of cell.

Within the lumen of the follicle, there is a hyaline material which stains deeply with eosin and which is called the colloid of the thyroid. In preparations made by the use of ordinary fixatives, (e.g. Bouins, formalin, etc.) the colloid is separated from the epithelium by peripheral vacuoles, which have been created by a contraction of the colloid in fixation. Colloid in sections prepared by the freezing-drying technique is free from such vacuoles. It is thus assumed that they are artifacts of preparation and that they are not present in the normal living gland (DeRobertis, 1941).

When a thyroid gland is called upon to produce larger amounts of thyroxine than it does in normal circumstances, it is considered an "activated" gland and the normal morphological histology is altered. A thyroid gland may be experimentally activated by exposure to cold or by the administration of thyrotropic hormone of the anterior pituitary gland. In such cases the colloid is diminished and there is an increase in the number of follicular cells by mitosis, as well as an increase in cell height. New follicles are not found during activation. The unity of the follicle seems to be determined by the connective tissue network surrounding it (DeRobertis, 1941). The central follicles contain the most active cells and the least active are the peripheral cells and those in the isthmus connecting the paired lobes.

The non-secreting gland is characterized by follicles distended with colloid which has been accumulated and stored. The epithelial cells become markedly flattened.

Of the number of common fixatives which are available for histological examination of the thyroid epithelium, Bouin's seems to be the most preferred. The staining method used most frequently is the hematoxylin-eosin combination which has the advantage of being both satisfactory and convenient. Other stains are, of course, available for special investigations, but those mentioned have the most favor for examination of the thyroid gland to determine qualitatively the state of activity based on height of the cells. The paraffin method is used almost exclusively as an embedding procedure.

There appears to be a good correlation between colloid volume and body size, and the colloid potency depends on the volume present, at least, as determined by a staining reaction (Hall and Kaan, 1942). Turner and Turner (1944) suggest a method of determining colloid volume by photographing sections of the thyroid gland and measuring follicular walls with a planimeter which eliminates the subjective selection of representative cells. Readings are obtained at once in terms of area, and these may be transferred to volume with the proper methods of computation involving the theory of sampling and known constants.

The method of measuring the height of a number of representative acinar cells with an ocular micrometer and determining an average, called the cell height "index", has come into fairly common use. Of the various techniques which have been introduced for this estimation, that of Rawson and Starr (1938) has been most widely employed. The height of a cell of average size in the wall of two hundred successive distinct acini is determined, the interacinar cells being neglected. These measurements are tabulated and a graph is made of the frequency curve and the mean and standard error of the mean are given. Using this tecynique, Uotila (1940) made one hundred measurements while Gorbman (1949) and others have used the height of fifty cells with satisfactory results. Dvoskin (1947) used the height of twenty-five successive follicular cells and found that the results were consistent within each group observed.

Griesbach and Purves (1943) suggested a modification of the above method in which a representative section from the center of the gland is projected onto a screen and thirty acinar cells were measured by means of a standardized celluloid scale prepared for that purpose. These workers (Griesbach and Purves, 1943) demonstrated that thirty measurements are all that are required for statistical accuracy and that additional measurements would be a waste of effort, for they would contribute no additional accuracy or reliability to the average value obtained for a gland.

The heights of cells which have been reported as normal in different strains of rats vary considerably. It is to be expected that there would exist differences among rats subjected to various external conditions, but the extremes of "normalcy" are surprising. The mean acinar cell height of the Sherman strain of rats reported by Cortell and Rawson (1944) was $3.86 \pm .47$ microns. Dvoskin (1948) reported that the average cell height in his normal control animals was 8.0 with a range of 7.8 to 8.6 microns, while the average height in thiouracil-treated animals was 13.5 microns.

MacKenzie and MacKenzie (1943) found that the hyperemia and enlargement of the thyroid gland in rats being fed goitrogens was accompanied by histologically observable reduction in colloid and an increase in height of the thyroid epithelium. These workers found that these changes could be prevented by the administration of exogenous thyroxine, and they suggested

that the thyroid enlargement was probably mediated through a hormone from the anterior pituitary body. This has been proved to be the thyrotropic hormone by a number of other researches. Uotila (1940) observed that the regulation of the thyrotropic function of the anterior pituitary depends primarily on humoral pathways with variation in the organism's thyroxine level as the most important factor, but that under certain conditions, such as exposure to cold, stimuli from the hypothalmus are transmitted through the pituitary stalk modifying the basal secretory rhythm of thyrotropin.

The morphological activation of the thyroid gland by a goitrogen and by exogenous thyrotropic hormone are identical in appearance. Since the activation of the thyroid by a goitrogen does not take place in the absence of the pituitary gland, it is then assumed that this activation is caused by thyrotropic action. Thyrotropic hormone thus appears to be the most conspicuous endocrine factor in the control of thyroxine, and it stimulates the thyroid gland to make its own hormone (Means, 1943). When potent goitrogens are present and sufficient exogenous thyroxine is not present for homeostasis, this morphological activation is futile since it cannot overcome the functional inactivation induced. Thus the thyroid gland becomes larger and has the histological appearance of an activated gland.

The ability of thyroxine to lower the height of the acinar epithelium has been repeatedly confirmed (Cortell and Rawson,

1944, and others). Thus it has been established that the functional level of the individual thyroid gland is indicated by its morpho-histological structure and that the height of the acinar epithelium is a means of estimating this level. This is valid except when an antithyroid substance is present which prevents the thyroid from manufacturing the thyroidally active substance.

When sufficient amounts of iodine are lacking for the gland to produce the thyroidally active substance, the effects are the same as when an antithyroid drug is present when hyperplasia and hypertrophy of the thyroid gland occurs as a result of thyrotropic stimulation (Astwood et al., 1943). In this case also the morphological activation is futile since it cannot overcome the functional inactivation in the absence of the required iodine.

It has been demonstrated that the acinar cell height is inversely proportional to experimentally altered environmental temperatures. Both the thyroid gland and the adrenal glands are in some manner concerned with the maintenance of a constant body temperature and these may operate in conjunction with the neural thermoregulatory mechanisms of the animal body. Adrenalectomized rats are unable to withstand the stress of low environmental temperatures and death occurs several hours post operatively due to decreased heat production (Bernstein, 1941).

The thyroid glands of rats kept in the cold develop hyperplasia, thickened acinar epithelium and the colloid loss

characteristic of a hyperactive thyroid (Bailif, 1937). This condition may be prevented, or at least lessened, by increasing the level of iodide in the diet (Kenyon, 1933) or by the administration of exogenous thyroxine (Turner, 1946).

Experimental procedures. Histological examination of the thyroid epithelium was made of normal and cold-treated rats of both sexes and strains. The rats were raised from parents which came from the colony of Hunt and Hoppert. These parents were mated and the young were born and cared for in a manner similar to those in the colony and under the same environmental conditions as to diet and general care.

All rats were killed by ether anaesthesia and their thyroids removed, cleaned of excess connective tissue and immediately placed in Bouin's solution for fixation. The paraffin technique was used, sections were cut at a thickness of seven microns and stained with Ehrlich's hematoxylin and eosin.

Examinations were made with the oil immersion lens of a standard microscope, and the height of the acinar epithelium was measured with the aid of an ocular micrometer. Twenty-five measurements, made in a systematic manner, were taken for the determination of the average height of the cells of each gland. Sections from the central portion of each gland were examined.

The instrumental measurements were used in the statistical analysis without converting them to microns. The method of analysis of variance was employed in analyzing the data.

For individual comparisons, rats of approximately equal age and sex were paired and then the resistant strain was compared with the susceptible strain. In addition, there was a comparison made on the basis of measurements of the individual cells since there were twenty-five such measurements made to determine a value for an individual rat.

Group I consisted of three female rats of each strain. The susceptible rats averaged188 grams in weight and the resistant rats averaged183 grams. These six animals were killed in the month of August.

Group II comprised seven males of each strain. The susceptible series rats had an average weight of 308 grams while the average weight of the resistant series rats was 295 grams. These fourteen rats were killed in the month of November.

Group III was made up of two females from each strain and they were killed in the month of May. These rats were kept alive in a refrigerator which was maintained at about 40° F. for six days prior to sacrifice. The thyroid weights of these rats, as well as body weights, were taken. The susceptible series body weights averaged 219 grams and the resistants 237 grams. The thyroid weights per hundred grams body weight for the four rats were slightly above the averages previously given for each series. The actual values of milligrams of thyroid weight per hundred grams body weight were as follows: susceptible series: 9.03 and 8.81 and resistant series: 6.02 and 7.54. The body weights of all four rats remained substantially unchanged during the cold treatment.

Experimental results. The thyroid follicles of the caries-resistant rats are, in general, smaller than are those of the caries-susceptible strain. Quantitative determinations were not made but this condition was evident from visual examination of histological sections prepared from the respective tissues.

Photomicrographs of representative sections of thyroid tissue are reproduced in plates 1, 2 and 3. Plate 1 shows sections from Group I females. Figures 1, 2 and 3 are sections from the thyroid glands of susceptible series rats, and Figures 4, 5 and 6 are from resistant series rats. The differences in the size of the follicles are obvious by inspection.

Plate 2, Figures 1-7 inclusive, are photomicrographs of normal group II susceptible series males. Figures 8 and 9 of this plate are from representative sections of cold-treated Group III susceptible series females. These figures may be compared with those of plate 3 where Figures 1, 2, 3, 5, 6, 8 and 9 are from representative sections of group II resistant series males. Figures 4 and 7 of this plate are from cold-treated group III females.

The differences in follicle size among the females of group I were also evident among the males of group II. The follicles of the susceptible series thyroid glands tended to be larger than did those of the resistant series glands. Examination of the thyroids of cold-treated animals of group III, however, shows little apparent difference between the two strains. The colloid area of the follicles of the susceptible

series thyroid glands appears to have been reduced as a result of the cold treatment. This suggests that there were different physiological responses in the two strains.

The frequency polygons of Figures IV and V show the distributions of the heights of acinar cells in each of the two strains. It is apparent from Figure IV, group I that the acinar epithelium of the resistant series rats was, on the average, slightly higher than was that of the susceptible series animals. While many of the cells were of about the same height, there were more high cells in the thyroid glands of the resistant series and more low cells in the susceptible series glands. This was also true in Group II as shown in Figure V.

The mean acinar heights for the three groups of rats of the resistant and susceptible strains are given in table 9. The standard error accompanies each mean. The mean height for each rat in each group with its standard error is also given. The standard error of the group means were calculated from the individual cell heights.

The mean acinar height for the three females of the susceptible series was 8.11 ± 0.24 microns; the comparable value for the resistant series was 10.22 ± 0.3 microns. The difference of 2.11 microns is not statistically significant when it is compared on the basis of the three rats' averages. There is evidence that group I contains more than one type of cell, however. When the six females are tested for homogeneity by the method of analysis of variance, the F value is 16.8 showing that there

is more than one type present. This F value is highly significant (Appendix V).

Since group I and II rats were killed at a different season and since they were not subjected to the stress of cold as were those of group III, they were maintained separately for statistical analysis. The data for group II rats are substantially in agreement with group I rats in the matter of differences between the two strains represented. The mean values representing the cell heights of both strains of rats are higher in this group than in group I. This confirms the observation made by Bernstein (1941) that the acinar cell height was greater in winter than in summer.

The mean acinar height of the susceptible series thyroids in group II was 11.19±0.25 microns and that for the resistant glands was 12.87±0.25 microns. The mean values of acinar cell heights of individual rats gave the results shown in Appendix V. The analysis shows that the difference of 1.68 microns is significant at the five percent level. As would be expected, a statistical treatment of the 350 individual cell heights in group II shows that there were two types of acinar cells (Appendix V).

The data of the cold-treated rats were analyzed in a similar manner (Appendix V). Since the difference between the two group means was only 0.35 microns (Table 9), which is a value close to the standard error of each mean, it is not significant. This shows that among the cold-treated rats no indications of differences were found.

TABLE 9

AVERAGE HEIGHT OF ACINAR EPITHELIAL CELLS (MICRONS)

Paired rat no. and group no.		Sex	Strain	
			Susceptible	Resistant
I	1	F	8.37±0.50*	10.83±0.40
	2	F	7.21±0.21	11.78±0.18
	3	F	8.74±0.39	8.05±0.32
	Average	F	8.11±0.24	10.22±0.31
II	1	M	12.61±0.56	12.10±0.48
	2	M	12.99±0.53	12.49±0.47
	3	M	9.96±0.53	12.77±0.50
	4	M	10.65±0.50	11.70±0.50
	5	M	10.26±0.47	12.86±0.42
	6	M	12.03±0.24	15.52±0.78
	7	M	9.90±0.19	12.63±0.74
	Average	M	11.19±0.25	12.87±0.25
III Cold treated	1	F	11.63±0.62	11.88±0.44
	2	F	10.18±0.45	9.23±0.25
	Average	F	10.91±0.39	10.56±0.32

* ± Standard error of the mean.

PLATE 1

Photomicrographs of Histological Sections of Thyroid Glands
Group I (females)

Figures 1, 2, 3 Susceptible Series

Figures 4, 5, 6 Resistant Series

The scale is shown in figure 6 where the vertical lines are 10 microns apart.

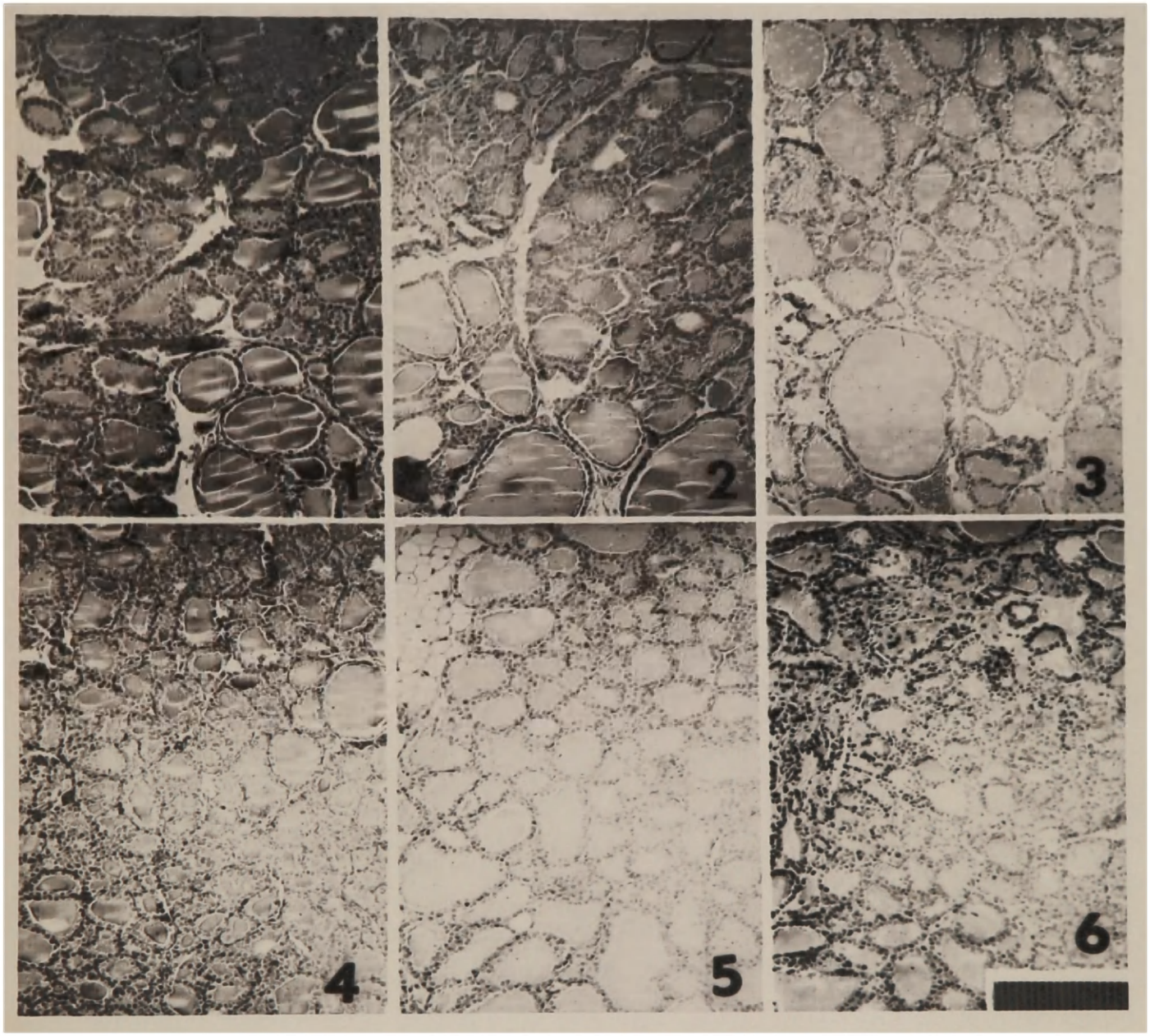

1
2
3
4
5
6

PLATE 2

Photomicrographs of Histological Sections of Thyroid Glands
Groups II and III Susceptible Series

Figures 1-7 Group II males

Figures 8-9 Group III females

The scale is shown in figure 9 where the vertical lines are 10 microns apart.

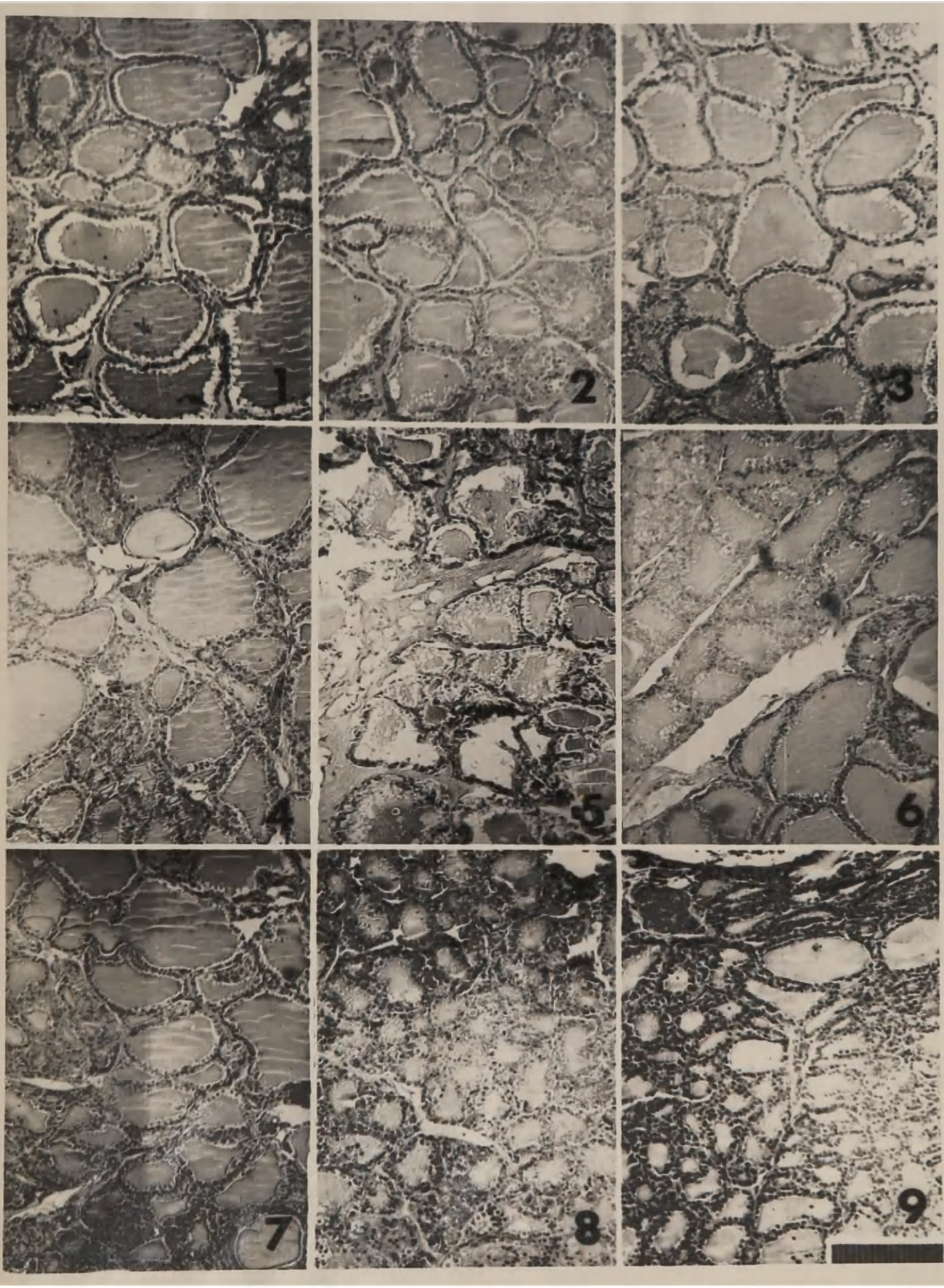

1
2
3
4
5
6
7
8
9

PLATE 3

Photomicrographs of Histological Sections of Thyroid Glands

Groups II and III, Resistant Series

Figures 1, 2, 3, 5, 6, 8, 9 Group II males

Figures 4, 7 Group III females

The scale is shown in figure 9 where the vertical lines are 10 microns apart.

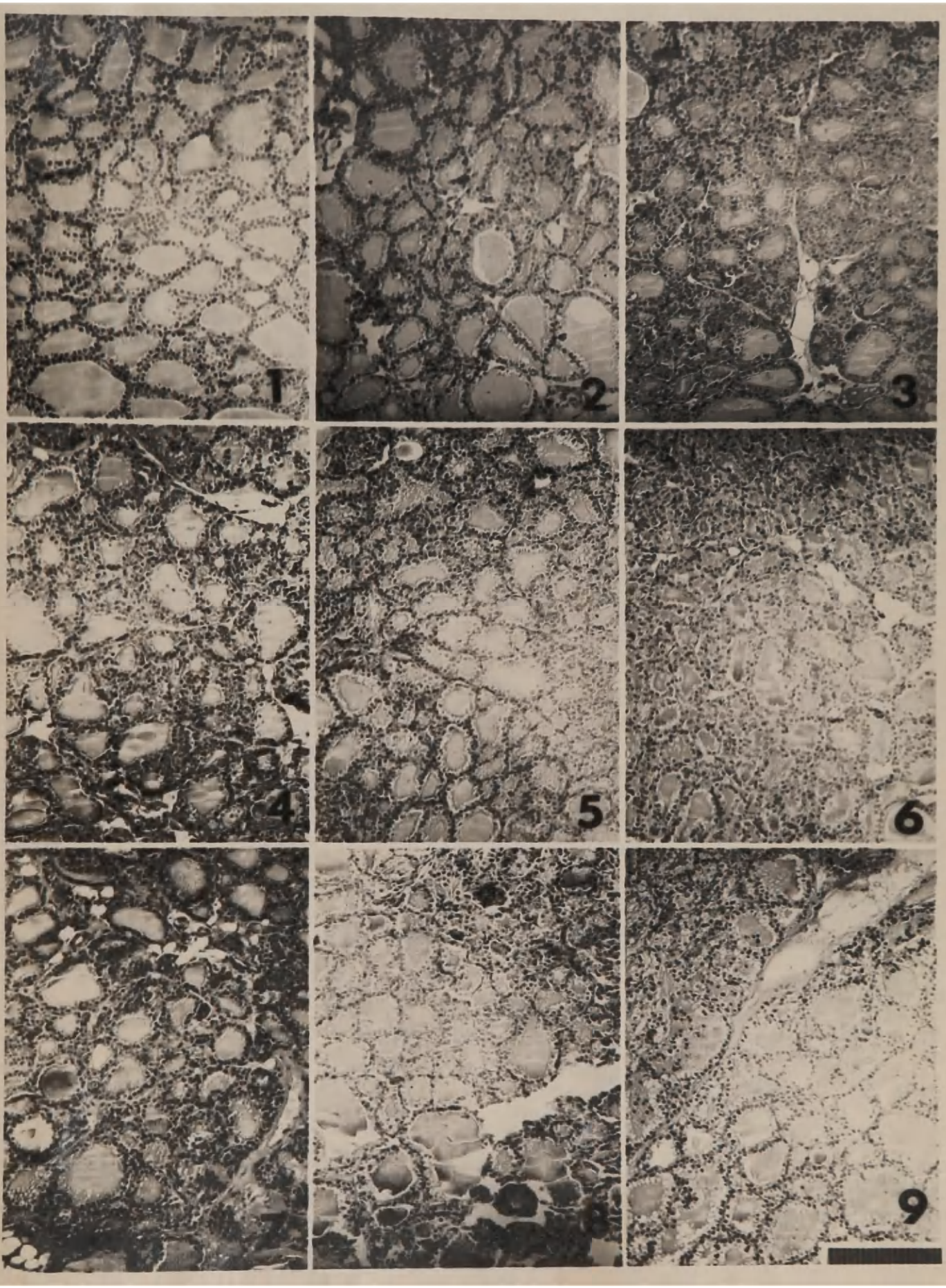

1
2
3
4
5
6
9

FIGURE IV

Relative Numbers of Acinar Cells of Various Heights

Group I (females)

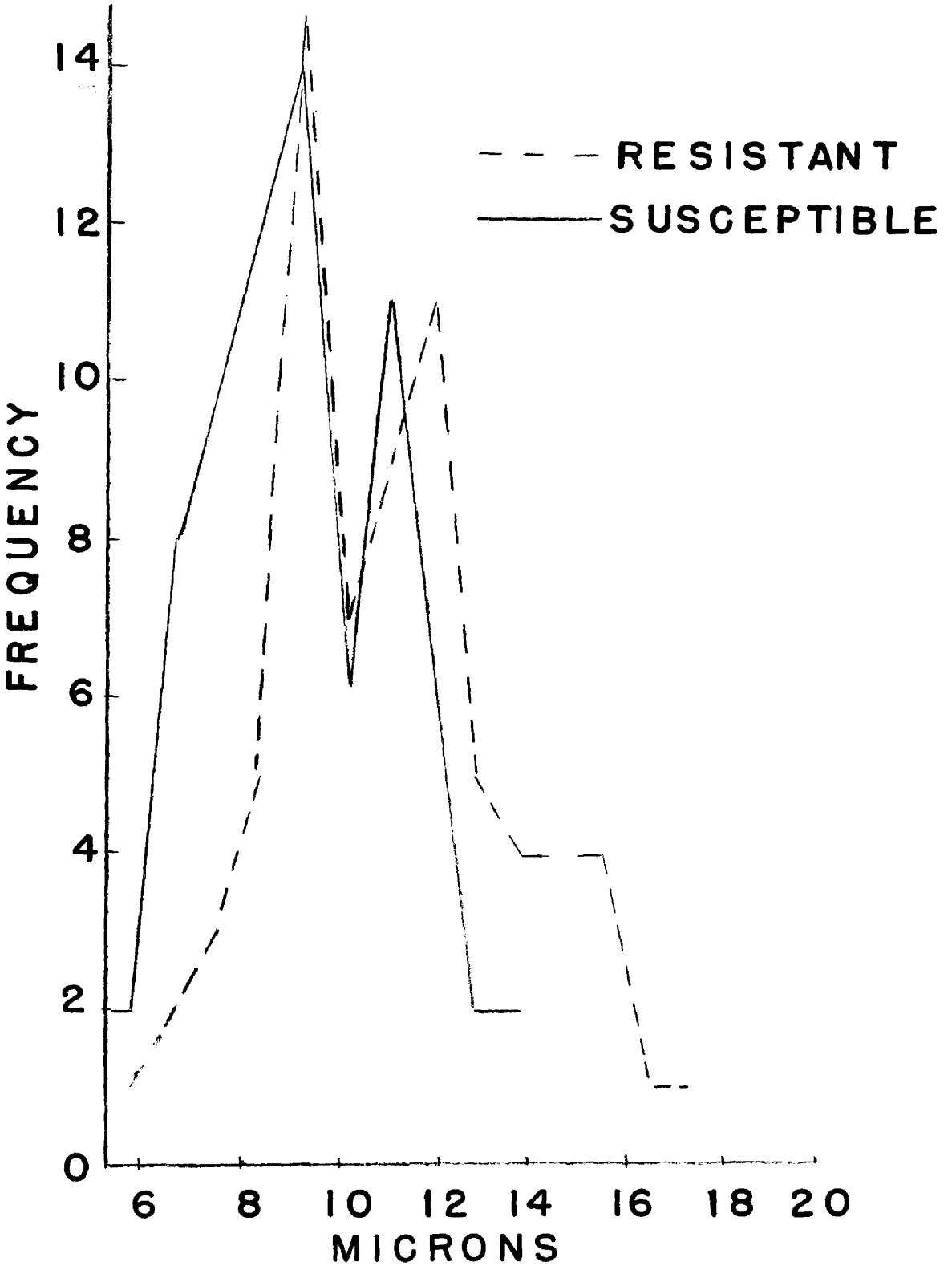
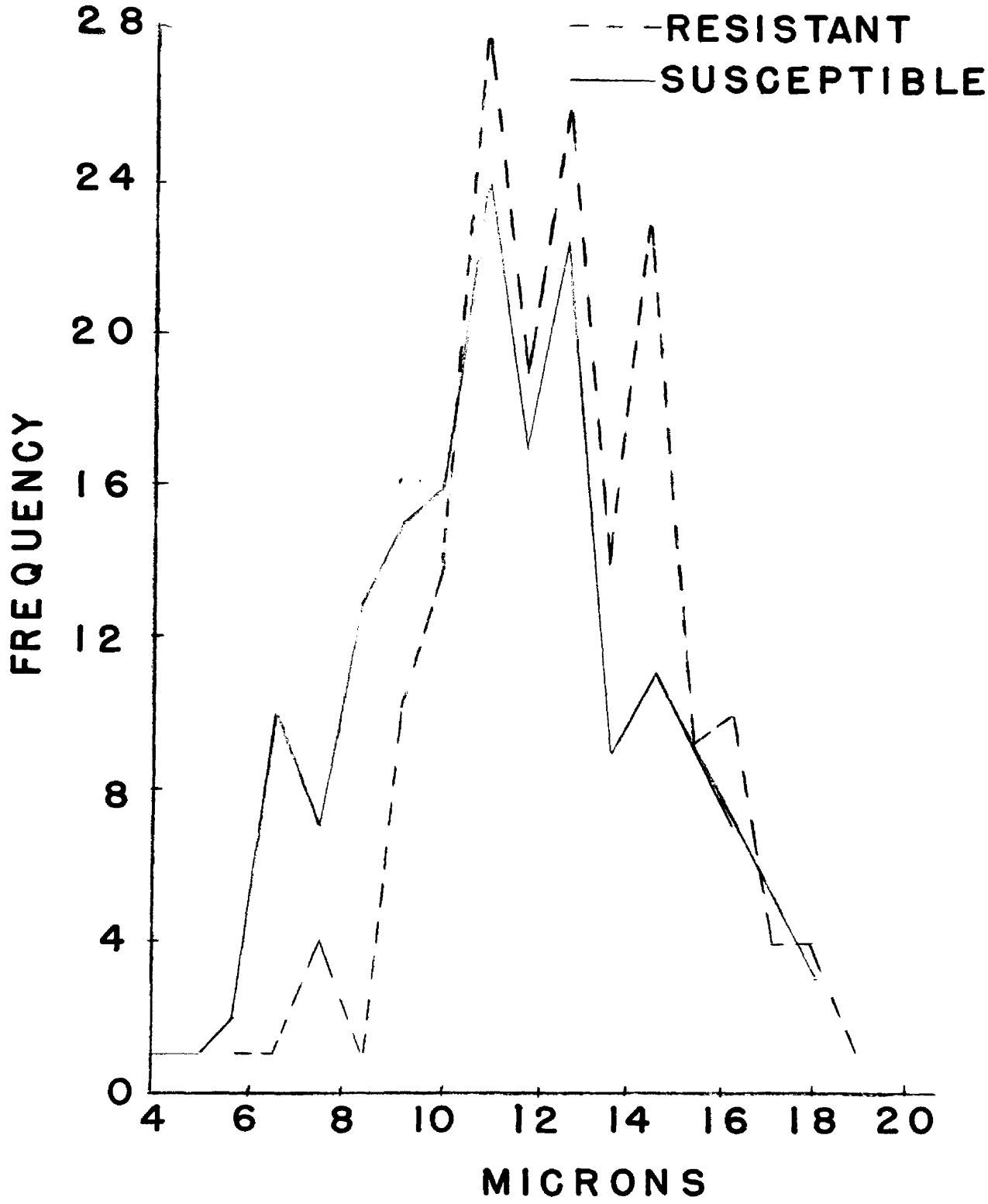

RESISTANT
SUSCEPTIBLE
FREQUENCY
0
2
4
6
8
10
12
14
6
8
10
12
14
16
18
20
MICRONS

FIGURE V

Relative Numbers of Acinar Cells of Various Heights

Group II (males)


RESISTANT
SUSCEPTIBLE
FREQUENCY
0
4
8
12
16
20
24
28
4
6
8
10
12
14
16
18
20
MICRONS

Conclusions. The thyroid follicles are larger in the susceptible series rats than are those of the resistant series. This could account, in part at least, for the heavier thyroid gland which is found in the caries-susceptible rats of the Hunt-Hoppert strains.

Measurements made of the height of the acinar epithelium of the thyroid glands of these strains show that the resistant series cells are higher than are those of the susceptible series. The higher cells indicate a greater function per cell in the resistant glands. The larger colloid area of the susceptible series glands indicates that there is more storage of colloid substance in these thyroid glands.

The thyroid gland of an animal which is exposed to cold becomes activated. This activation results in a diminution of colloid and an increase in height of the follicular cells (Monroe and Turner, 1946). This response appeared to have been obtained in the susceptible series females where the normal cell height was 8.11 μ and the cold-treated thyroid cell height was 10.91 μ. The amount of the colloid was also diminished as is shown by comparing plates 1 and 2 and 3. Neither of these responses, however, appear evident in the thyroids of the resistant series rats where there was little change either in colloid or cell height. It would seem then, that the resistant series rat was able to adjust its metabolism in response to the cold treatment without cytological alteration of the thyroid gland. The thyroid gland of the susceptible series rat, however, became activated in response to the cold treatment.

The threshold of response to cold treatment seems to have been reached in the susceptible series rats but not in those of the resistant series.

IV. SUMMARY

Physiology of the thyroid. It has been demonstrated that there are significant differences in the weight, in the uptake of radioactive iodine, as well as in the normal histological appearance of the thyroid glands of the Hunt-Hoppert strains of rats. It was not possible to demonstrate any significant differences in gaseous metabolism. If there were any thyroid secretion rate differences, they would be expected to be shown in metabolism variations. Since there were none, apparently the thyroid secretion rats is about the same in both strains of rats. The thyroid glands of the caries-susceptible rats are approximately 43 percent larger and show about 42 percent slower turnover of I^{131}. If the thyroid iodine content of the susceptible rats is increased in proportion to their weight, the net output daily would be equal in the two strains of rats. Unfortunately iodine analysis of the thyroids are not available. It appears, however, that the thyroid mechanism has been compensated in the susceptible strain so that the net output is metabolically equivalent to that of the resistant strain. This compensation resulted in a larger gland with more colloid.

The relatively low total uptake of radioiodine by the thyroid glands may be attributed to the fact that the rats were being fed an iodine-rich diet.

It has been shown that the thyroid secretion rate of the resistant series rats is somewhat lower than values reported by other investigators using other strains of rats.

There appeared to be no difference in the weight of the pituitary glands of rats of the two strains.

Genetics. There are genes for caries resistance and caries susceptibility. There are also genes for the various characteristics involving the thyroid which have been studied here. The weight of the gland, thickness of the epithelium and the amounts of colloid were different in the two strains. There were also differences in retention of radioactive iodine. The strains of rats were fairly uniform in these respects. Gross differences were present between the strains. Perhaps the same genes responsible for thyroid characteristics also affect caries, or perhaps the thyroid genes may be linked with the caries genes. Selection has been attempted to sort out and fix the caries genes, but no conscious effort has been made to do this for the genes effecting the thyroid gland.

The selection preferences for good fertility, rapid growth and generally good condition were about equal for the two strains of rats. This selection would not tend to sort the genes for caries and those for thyroid physiology into separate lines as has been done. It would thus seem logical to conclude that the inherited differences in thyroid physiology are either closely linked to or share common genes with the inherited factors in tooth decay.

Parotid gland. It has been demonstrated that the inherited differences for the production of caries between the caries-susceptible and caries-resistant strains of rats are not due to

the secretions of the parotid gland. Caries were neither inhibited nor were they markedly accelerated in the absence of secretions of the parotid gland.

APPENDIX

I. Caries times for parotidectomized rats and their intact sibs.

II. Tables of I^{131} recovery.

III. Average weights of the thyroid glands among the groups.

IV. Tables of age and analysis of variance of respiratory metabolism.

V. Tables of analysis of variance of acinar cells.

VI. Vitamineral supplement analysis.

APPENDIX I

CARIES TIME FOR PAROTIDECTOMIZED RATS AND THEIR INTACT SIBS

Susceptible Series

Cross	332		358		360			361		
Rats	Colony	Exp.	Colony	Exp.	Colony		Exp.	Colony		Exp.
Caries time	27	46	none	43	41	77	67	43	29	41
	43	47		28	27	63	67	43	29	44
	27	47		28	41	77	39	29	29	40
	27	90		28	27	49		43	29	54
	27			28	83	49		43		
	27			43	27	49		43		

Cross	362		365			367			368	
Rats	Colony	Exp.	Colony		Exp.	Colony		Exp.	Colony	Exp.
Caries time	75	35	39	26	54	38	36	35	38	41
	47	35	39	26	40	38	29	91	53	41
	47	63	39	26	69	50	29			56
	47	49	39	26	55	22	29			41
	91	49	39	26	55	36	29			56
			39	26	55	52	43			
			39	26	67	36	59			
					55					

Cross	369			371		373		
Rats	Colony		Exp.	Colony	Exp.	Colony		Exp.
Caries time	31	31	43	24	33	39		71
	43	57	43	24	33	39		140
	57	31	44	24	19	53		99
	31		44	24	46	39		71
	31		72	24	33	39		
			58	24	33	39		
				24	46	39		
					46			

CARIES TIME FOR PAROTIDECTOMIZED RATS AND THEIR INTACT SIBS (cont.)

Resistant Series

Cross	501		506		508		509	
Rats	Colony	Exp.	Colony	Exp.	Colony	Exp.	Colony	Exp.
Caries time	202 346	119	232 385	393	222 700	221	718 446	120
	420 301	163	110 665	703	397 279	283	388 614	120
	119 170	105	544 681	326	291 159	221	264 538	
	466 225	105	477 518	483	249 685	415	566 477	
	613 436	163	647 546	552	291 355	542	482 752	
	480 286	189	709 616		220 428		614 646	
	480 321	119					482 660	
	480 196	163					496 477	
	201	163						

Cross	510		520		521		523	
Rats	Colony	Exp.	Colony	Exp.	Colony	Exp.	Colony	Exp.
Caries time	516 547	243	677	601	477	876	476	853
	377 543	119	795	631	619	700	566	281
	88 381	154	592	858	682	477	824	808
	421 338	304	419	858	695	508	887	712
	290 471		755	641	562		606	
	290 353		207		774		387	
	305 472		549		774		238	
	305 443		710				668	
	465 505						749	
	501 344						491	
	674 268						304	
	325 719						435	
	289 574						421	
	355 558							
	473							

Cross	525		533		534		543	
Rats	Colony	Exp.	Colony	Exp.	Colony	Exp.	Colony	Exp.
Caries time	703 783	619	431	511	487 239	134	420 612	516
	214 704	517	234	384	277 304	232	897 689	821
	437 486	134	279	436	563 621	548	685 554	669
	584 783	813	220	529	390 199	490	789 701	526
	626 734	619	270	535	430 318	223	441 527	516
	774 691		417	560	318 507		441 743	
	569			451			480 126	
							698	

APPENDIX II

COUNTING RECORDS OF I^{131} RECOVERY

Trial I

	18 hr. no. and series		68 hr. no. and series	
	(5)* susc.	(9) res.	(6) susc.	(9) res.
Counts per sec. per thyroid	38.97**	34.53	45.37	24.87
	5.18	7.67	3.00	3.67
Counts per sec. per 100 gm. B.W.	27.91	27.96	34.65	17.74
	4.61	5.63	3.10	3.13
Counts per sec. per mg. thyroid	3.84	3.48	4.21	3.04
	1.56	0.47	0.26	0.37
Percent of administered dose present at sacrifice	4.65	4.25	5.41	2.97
	0.62	0.28	0.36	0.44

Trial II

	68 hr. sacrifice, sex series and no. of rats			
	(12) susc.F	(20)susc.M	(15) res.F	(7) res.M
Counts per sec. per thyroid	55.88	46.44	33.60	33.50
	4.68	2.79	1.83	2.97
Counts per sec. per 100 gm. B.W.	41.92	46.44	18.04	8.30
	1.87	0.64	1.16	1.12
Counts per sec. per mg. thyroid	4.07	2.52	3.28	2.45
	0.29	0.28	0.17	0.72
Percent of administered dose present	6.34	5.27	3.82	3.80
	0.53	0.31	0.23	0.37

APPENDIX II (cont.)

Trial II (cont.)

	148 hr. sacrifice, sex series and no. of rats			
	(12) susc.F	(24)susc.M	(16)res.F	(7) res.M
Counts per sec. per thyroid	43.96	40.07	24.20	25.15
	3.06	1.62	1.72	1.18
Counts per sec. per 100 gm. B.W.	30.51	16.28	13.24	8.87
	2.00	0.94	0.86	0.43
Counts per sec. per mg. thyroid	3.47	2.01	2.22	1.82
	0.20	0.13	0.17	0.13
Percent of administered dose present	4.99	4.50	2.75	2.85
	0.35	0.18	0.19	0.12

Trial III

	68 hr,sex series, and no.		148 hr,sex series, no.	
	(12)susc.F	(8) res. M	(13)susc. F	(9) res.M
Counts per sec. per thyroid	51.95	33.41	41.96	23.87
	5.80	2.65	1.74	1.13
Counts per sec. per 100 gm. B.W.	32.70	10.74	26.25	8.02
	4.20	1.02	1.68	0.38
Counts per sec. per mg. thyroid	3.83	1.95	3.01	1.54
	0.34	0.18	0.18	0.06
Percent of administered dose present	5.39	3.46	4.35	2.53
	0.61	0.33	0.61	0.11

* Number of rats
** Each value shown with its standard error.

APPENDIX III

Average Weight of the Thyroid Gland among the Groups

Susceptible Series (males, M; females, F)

Group	Sex	No. of rats	Thyroid Wt. per 100 gm. B.W.
1	F	12	8.84±.32*
2	F	12	8.81±.36
3	F	12	8.15±.49
4	F	13	8.79±.38
Sum	F	49	8.58±.19
1	M	20	7.06±.25
2	M	24	8.15±.25
Sum		44	7.65±.20

t sexes = 1.12

Resistant Series

Group	Sex	No. of rats	Thyroid Wt. per 100 gm. B.W.
1	F	15	5.54±.20
2	F	17	6.04±.18
Sum	F	32	5.62±.22
1	M	7	5.00±.43
2	M	7	4.93±.25
3	M	8	5.54±.30
4	M	9	5.22±.21
Sum		31	5.19±.16

t sexes = 0.50

* Standard error of the mean.

APPENDIX IV

Mean Age and Body Weights of Rats Used for Respiratory Metabolism Determinations

Strain	Run	Age, days	B.W. gm.
Susceptible	1	347±0*	348±9*
	2	292±8	308±18
	3	123±1	258±18
Resistant	1	330±3	374±7
	2	334±7	358±15
	3	105±0	255±9

* Standard error of the mean.

Analysis of Variance, cc. O_2 per $\left(\frac{BW}{100}\right)^{.73}$

Source	D.F.	S.S.	M.S.
Strains	1	553.43	553.43
Individual rats	34	6,514.88	191.614
Total	35	7,068.31	

F. = 2.89, t = 1.70 (not significant)

Analysis of Variance, cc. O_2 Consumption per 100 g. B.W.

Strains	1	576.8	567.8
Individuals	34	4,865.5	143.1
Total	35	5,442.83	

F. = 3,949, t = 1.98 (not significant)

Analysis of Variance of the Runs and the Chambers (using O_2 per $\left(\frac{BW}{100}\right)^{.73}$ values)

Chambers	11	1,663.20	151.20
Runs	2	564.73	282.36
Discrepance	22	4,840.38	200.17
Total	35	7,068.31	

Chambers, F. = 0.75 (not significant)

Runs, F. = 1.416 (not significant)

Analysis of Variance of Induced Hypo- and Hyperthyroidism

Source	Degrees of Freedom	Sum of Squares	Mean Square
Sexes	1	250.0	250.0
Treatments	3	63.10	21.03
Interaction	3	592.68	197.56
*Individuals	30		194.44

* From an analysis of the original data

F. sexes = 1.25

F. treatments = .11

F. interaction = .99

APPENDIX V

Analysis of Variance of Acinar Cells of Group I (Measurements)

Source	Degrees of freedom	Sum of squares	Mean square
Total	149	126,417.3	
Groups	1	20,230.4	20,230.4
Measurements	148	106,186.9	717.5

F = 28.2 (highly significant)

t = 5.3 (highly significant)

Analysis of Variance of Acinar Cells of Group I (Mean Values, Paired)

Source	Degrees of freedom	Sum of squares	Mean square	F
Pairs	2	2.15	1.08	.26
Strains	1	8.09	8.09	1.92
Discrepance	2	8.43	4.22	
Total	5	18.67		

F pairs = 0.26 (not significant)

F strains = 1.92 (not significant)

(The discrepance term was used as the error term.)

Analysis of Variance of Acinar Cells of Group I

(Mean Values, not Paired)

Source	Degrees of freedom	Sum of squares	Mean square
Means	5	46,650.3	9,330.1
Measurements	144	79,767.0	553.9
Total	149	126,417.3	

$F = 16.844$ (highly significant)

The value $F = 16.8$ indicates that there is significant variation present and that the cells do not constitute a single population.

Analysis of Variance of Acinar Cells of Group II

(Mean Values, Paired)

Source	Degrees of freedom	Sum of squares	Mean square
Pairs	6	136,067.4	22,677.9
Strains	1	117,394.5	117,394.5
Discrepance	6	99,281.5	16,546.9
Total	13	352,743.4	

F strains = 7.09 (significant)

Analysis of Variance of Acinar Cells of Group II

(Measurements)

Source	Degrees of freedom	Sum of squares	Mean square
Rats	6	34,394.5	5,732.4
Strains	1	29,679.3	29,679.3
Interaction	6	24,691.1	4,115.18
Measurements	336	331,491.3	986.58
Total	349	420,256.2	

(a) On the basis of measurements as the error term:

F rats = 5.81
F strains = 30.08 (highly significant)
F interaction = 4.17

(b) On the basis of interaction as the error term:

F rats = 1.39
F strains = 7.21 (significant at .05 level)

Analysis of Variance of Acinar Cells of Cold-Treated Rats (Mean Values Paired)

Source	Degrees of freedom	Sum of squares	Mean square
Pairs	1	509.15	509.15
Strains	1	15.21	15.21
Discrepance	1	71.08	71.08
Total	3	565.02	

F strains = 0.21 (not significant)

Analysis of Variance of Acinar Cells of Cold-Treated Rats (Measurements)

Source	Degrees of freedom	Sum of squares	Mean square
Rats	1	12,633.8	12,633.8
Strain	1	376.4	376.4
Interaction	1	1,102.2	1,102.2
Measurements	96	61,696.6	642.67
Total	99	75,809.0	

(a) On the basis of measurements as the error term:

F rats = 19.66
F strains = .586
F interaction = 1.71

(b) On the basis of interaction as the error term:
F rats = 11.46
F strains = .34 The F values are not significant.

APPENDIX VI

Vitamineral Supplement Analysis

Calcium	not more than	30.0000%
	not less than	28.0000%
Phosphorous	not less than	5.0000%
Manganese	not less than	0.1400%
Iodine	not less than	0.0425%
Iron	not less than	0.1700%
Salt, sodium chloride		none

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