THE PHYSIOLOGY OF THE PARATHYROID GLANDS 1916

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There are in the animal organism a number of epithelial bodies occupying definite anatomical positions. Some of these structures, which we call glands, possess duct systems that direct the product of metabolism of the epithelia into various body cavities, where this product or secretion carries on a definite physiological activity. On the other hand, a number of these epithelial bodies are without ducts so that no products of their metabolism can be directly collected. These are the ductless glands and because of the intimate relationship between these cells and the lymph and blood capillaries, these glands have been supposed to elaborate substances, which are then carried by the blood or lymph streams to the various cells of the body. They have been named, therefore, the glands of internal secretion. The question of their physiological significance has interested the biologist for many years. Yet up to the present day, very little light has been thrown upon the mechanism of their activities.

A brief reference to their phylogenesis will show that they are specializations of protoplasm, developed so as to better serve the requirements of the cells of the complex multicellular organism. Naturally, with the development of the organism from a simple single cell to a much larger structure composed of millions of cells, which may be widely separated and therefore placed in different environments, these variously located cells must not only carry on the vital processes of their simple progeniture, but also according to the principle of adaptation, must specialize in those activities which their environment requires for their best service to the organism. Thus the surface cells become protective; certain other cells become highly contractile and accomplish locomotion. Now as a result of this specialization in function, the generality of function, as found in the unicellular organism, must be to some extent sacrificed. And the specialized cell becomes dependent, perhaps parasitic, upon the other cells for a portion of their activities, which the specialized cell no longer finds the opportunity to carry out itself; thus the principle of mutual service is developed. And it may be assumed that the glands of internal secretion, by the nature of their relationship to the blood stream, use this medium to supply vitally important substances to the other cells of the body. We find a concrete example of this in the chemical structure of the thyroid apparatus. In sponges, which have no thyroid gland, considerable iodin is found in the skeletal structures. In the higher organism, fishes, mammalian, etc., there is a thyroid gland containing considerable iodin whereas the skeletal structures contain it no longer. It may be assumed, then, that the thyroid gland with its iodin compound does that metabolic work for the skeletal structures, which they once performed themselves when they contained the iodin compound. Confirmation of this assumption is observed in diseases of the thyroid apparatus where abnormalities in the skeletal tissues result. The physiological relation of adrenaline, found in adrenal glands, to the sympathetic nervous system further substantiate the interdependence between the services the glands of internal secretion may offer to those other tissues, which now work more directly in the adaptation of the organism. Using the above assumption as a guide, this writer has endeavored to elucidate the mechanism of activity of the parathyroid glands, which up to the present research, though given much attention, has remained relatively obscure.

WORKING BASIS AND HISTORICAL SKETCH

The thyroid glands are two oval bodies located ventrally and laterally to the thyroid cartilage and trachea in the neck. In the neighborhood of these larger glands, or imbedded in them, are four small epithelial bodies. These are the parathyroids. They may be grouped in two pairs: the superior pair being located about the superior poles of the thyroid, the inferior pair generally at the inferior poles. They are flattened, round, or oval in shape and in the dog, together present a mass no larger than a lentil. Because of their small size and irregular occurrence, they were not discovered until 1880, when Sandstrom called attention to their individuality.

Histologically, their structure differs greatly from that of the thyroid with which they are so closely associated. They receive their blood supply from the branches of the thyroid artery. Their veins enter into those of the thyroid. The glands have a capsule of connective tissue, which supporting the larger vessels dips into the glandular substance, imperfectly separating it into lobules. These lobules may take the form of columns of cells, which anastomose; these columns may be made of one or several rows of cells that sometimes group into round follicles. There are three varieties of cells uniformly observed. Two of these types are larger than the thyroid cells having deeply staining nuclei and slightly staining protoplasm with fine boundaries that are quite distinct. The other type resembles the thyroid cell, being low columnar and arranged on a basement membrane which surrounds, follicle-like, a small lumen that may be filled with a granular or colloidal material. Of the former type, a minority of the cells are large polygonal with a deeply staining nucleus and deeply staining granular protoplasm, which because of its acidophil nature resembles some of the hypophysis cells. These several varieties of cells may perhaps represent, by their differences in appearance, various stages in functional activity. Very striking is the richness of the blood supply. This is of the sinusoidal type. The thin endothelium of the blood capillaries is placed in close apposition to practically the entire surface of each cell, from which it is not even separated by fibrous tissue strands. Frequently, glands are found in which the epithelial cords are split by the accumulation of colloidal material and thus, an acinus is developed which resembles closely the follicles that make up the thyroid gland. For this reason, the parathyroids have been looked upon for sometime as embryonic thyroids.

Whether they have a physiological significance differing from that of the thyroids was disputed for sometime, since their supposedly complete removal from the animal's body was not always followed by definite physiological changes. This is not surprising for a complete surgical removal of the glands is sometimes impossible. Occasionally, one of the glands may be found in relation to the thymus, or in some other part of the mediastinum. We have learned, moreover, through the work of Halsted that only a minute portion of parathyroid tissue is capable of rapidly re-growing to a sufficient size to carry on the work of all the glands. This was proven in an experiment by carefully removing all the glands that could be found, and then transplanting a small piece of parathyroid tissue into the abdominal wall. It was found that with this small piece of grafted parathyroid tissue, the animal behaved quite normally for a long time. After the transplant was removed, however, the typical complex of symptoms developed, which characterizes the parathyroid insufficiency. It is not surprising, therefore, that the older observers (Forsyth) looked upon the parathyroids as incompletely developed accessory thyroids, supporting this view with the observation that after removal of the thyroids, the parathyroids rapidly changed their structure to resemble that of the thyroids. In order to explain the frequent appearance of the syndrome of parathyroid insufficiency, they assumed that such changes were brought about by injury to the superior laryngeal nerve, or other tissues of the neck region (Munk).

The capacity of the parathyroid to take up the thyroid's function does not exclude, however, an independent significance; that these glands mean something more than the thyroids was shown by Beidl, Moussu,2 Glay,3 Vassale, and Generale4 when they pointed out that the removal of the thyroid produces a condition of cachexia as well as changes associated with myxedema, whereas, removal of the parathyroids is responsible for a typical nervous symptom complex.

This syndrome, though often referred to as typical, has been rather incompletely described in the literature. Several detailed protocols are, therefore, submitted. The behavior of the parathyroidectomized dog may coincide with either of two distinct types of symptoms, or with a mixture of these types, in which either symptom may predominate. In one type, the dominant feature is over excitability, in the other, under excitability. In the former, tonic convulsions are characteristic; in the latter, we observe a peculiar muscular flaccidity and a general depression of the nervous system. In either case, a pathological condition develops between one and a few days following the removal of the glands and proves fatal within two to ten days after surgery. The first type is illustrated in the following protocol:

Dog (3) was completely parathyroidectomized on December 6, 1913, at 7 P.M. At 8 A.M., December 7th, he had recovered from all visible effects of the anesthetic and operation. He seemed fairly bright and active until 11 A.M., December 8th. At this time the first symptoms of tetany were noticeable in a wrinkling of the forehead and twitching of the right ear. At 12 o'clock the twitching was visible in the shoulder muscles and the hind limbs were somewhat extended. Also during this time the heart rate had increased to 160 per minute; the respiration became more rapid and somewhat labored. At 2 P.M., the dog was found in complete tetany, lying on his side with limbs extended and with opisthognathous, as the jaws were locked and the hips raised. The eyes, because of the retraction of the lids, seemed to bulge out, the pupil was dilated and the sclera injected.

Salivation and lacrimation were profuse. During this convulsion the respirations were extremely difficult, each inspiration and expiration producing a sound suggestive of laryngeal spasm. Inspirations were deep and expirations seemed incomplete and difficult. At about 2:15 P.M., the dog attempted to rise to his feet, but his struggles were futile since flexion of the limbs was impossible. By 2:50 P.M., the respirations had become shorter and more rapid with the production of less sound and it was noticed that although the hind legs were extended, one of the fore limbs was now flexed and instead of being in general toxic spasms, the muscles now played in twitches. A similar twitching was also observed in the shoulder and trunk muscles. By 3:30 P.M., the respiration changed to a rapid panting, and the muscles of the fore limbs were more flaccid, while those of the hind limbs exhibited twitches. The face still retained a peculiar expression with raised lips, wrinkled nose and brow, and the ears were drawn back. He rested in this condition until about 7 P.M., when another and similar convulsion developed which did not subside until after midnight. At 6:30 A.M., the subject was found dead in the cage in an attitude of opisthotonus. Necropsy showed a hyperemia of all of the viscera. The ordinarily invisible intestinal vessels were so dilated as to be easily traceable, the liver and spleen were markedly congested, the bladder filled with urine, and the intestinal tract with fluid.

The most striking histological changes occurred in the blood, liver, kidney, and brain. The blood of the vena cava and of the heart, in this as well as all the other animals, showed extensive ante-mortem coagulation. White clots, in several cases, were continuous from within the heart chambers down the vena cava to its iliac bifurcation. They nearly filled the lumen of the vessel. Upon section of the liver, the vessels showed fragmented erythrocytes, many normoblasts, erythroblasts with mitotic nuclei, and a small proportion of erythrocytes that stained brilliantly in eosin; the remaining red cells, in the large areas, were blood shadows. Each section of the liver and lung showed a number of large mononuclear cells with eosinophils granules. There were also present a larger number of large, flat, cells staining very intensely in eosin. These showed no definite granulation. In places, they were found to line the smaller veins like the endothelial cells. In these places, no endothelial cells could be observed. The cells of the hepatic cords showed advanced, fatty, degeneration of the protoplasm. The nuclei of the large areas had disappeared entirely in places where the cell’s form was fairly well preserved. These areas were surrounded by circular areas of cells, in which the nuclei had become densely, stained, clumps of chromatin. In the livers of four of the dogs, only a diffuse chromatolysis could be observed.

All kidneys showed marked congestion and hemorrhage in the cortex, some anemic, and others congested medullae. Some glomeruli had lost Bowman’s capsule and were hemorrhagic, while others were markedly congested. In some of the convoluted tubes, the epithelium had degenerated.

The spleen contained a large quantity of pigment. Some of the cells showed chromatolysis. The lung showed edema, congestion, along with the blood changes already mentioned. The brain sections, which I prepared in Professor Barrett’s laboratory, showed cells in the motor areas with partial loss of Nissi substance, along with the typical tetany nuclei. Various degrees of chromatolysis were also observed in these nuclei.

Besides the intestinal tract, marked congestion also showed in the duodenum and the pyloric end of the stomach with disintegrating epithelial cells. Their nuclei were converted into solid, deeply, staining clumps. These appeared, like those in the process of extrusion, from the normoblasts.

It is observable that the totality of the symptoms point to a hyper-excitability of the whole nervous system, including those neurons contributed by the cord to the sympathetic system. Now the action that may elicit so striking a positive phase, may also be expected to present a negative phase, in which a general depression of the central nervous system results. Out of 47 dogs, 2 such cases were found. The following protocol will illustrate:

Dog (31) was operated upon March 8, 1914 at 2 P.M. It recovered from the anesthetic and was apparently normal until March 10th, when instead of walking about the cage and welcoming its attendant, it exhibited no recognition of his presence. It was examined and found lying asleep in a peculiar state of flaccidity with limbs somewhat flexed. When a limb was moved or the head turned back, it retained the attitude given it. The subject was not observed to move during the day. It remained in this condition until March 12th when, at 2 P.M., it was found dead in the attitude of dogs that die in tetany. A postmortem examination revealed no signs of pneumonia or any other infection. We, therefore, believe that the dog succumbed to parathyroid insufficiency. This case presents, perhaps, an over stimulation of the central nervous system, comparable to shock, a state which may be compared, perhaps, to the reversing of a chemical reaction by the products of the reaction.

The regular occurrence, after a complete parathyroidectomy, of a typical symptom complex facilitates the study of the mechanism by which these glands functionate, since a study of the causation of the tetany should reveal the position of these glands in the metabolism of the organism. Previous to this research, only one contributing fact has been brought forth; it was MacCallum’s discovery that the urine of parathyroidectomized animals contain excessive quantities of calcium, and that when calcium salts are injected intravenously into such animals, the tetany is immediately controlled. Therefore, MacCallum6 expressed the view that the parathyroids had to do with the metabolism of salts, but more especially the calcium salts; he referred to this substance a special physiological value, such that when it was lost from the animal’s body, a calcium deficiency resulted that constitutes the essential, pathological, condition of parathyroid insufficiency. It was shown, by Beebe7 and Beebe and Berkeley, that injections of other salts have similar, though not so marked an effect. These observers showed that the length of time over which aqueous solutions of calcium or other salts are useful in controlling the tetany is relatively short, varying between one and several days. It appears that if calcium insufficiency were the essential change, the addition of calcium to the body through intravenous injection should alleviate the pathological condition, so long as this treatment was used.

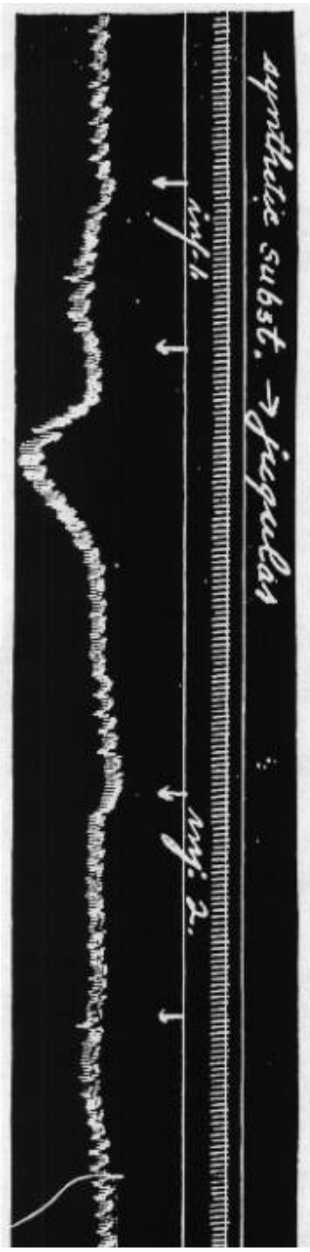
In a recent investigation, this writer found that when the tetany became uncontrollable through injections of aqueous salt solutions, the kidneys had become so pathological as to be unable to functionate normally. Since one of the effects of such intravenous injections is as a diuretic aiding in the elimination of toxic substances from the blood, it may be assumed that one of the beneficial effects of the aqueous calcium injections is dependant upon increasing the work of the kidneys and thus, the detoxification of the blood. That calcium should herein be more valuable than the other salts may depend upon its depressing qualities, but the fact that it is a diacid base would indicate its value as an acid carrier to be greater than that possessed by the monovalent metals. Therefore when excessively excreted from the body, a large number of acid radicals are lost, and when injected, a large content of acid radicals are added to the blood. If then the value of calcium depends upon the increasing or maintaining of a certain reaction of the blood, the acid radicals are here the important factors. They present two possible modes of activity: the simple neutralization of basic substances excessively elaborated within the body, or the destruction of such substances as are capable of producing the tetany.

There is still another source of indications that aid in the directing of the present investigation. We expect that when a vital process is removed from the organism, the dependent processes will come to a standstill. If this is true, some hitherto useful substance should be present and excreted from the organism, which goes unused as fast as it is offered, for the metabolism. Such a substance must contain vitally reactive groups and if these groups are not taken care of in the normal manner, they present the possibility of disturbing other vitally reactive substances within the organism and thus, the potential to become toxic.

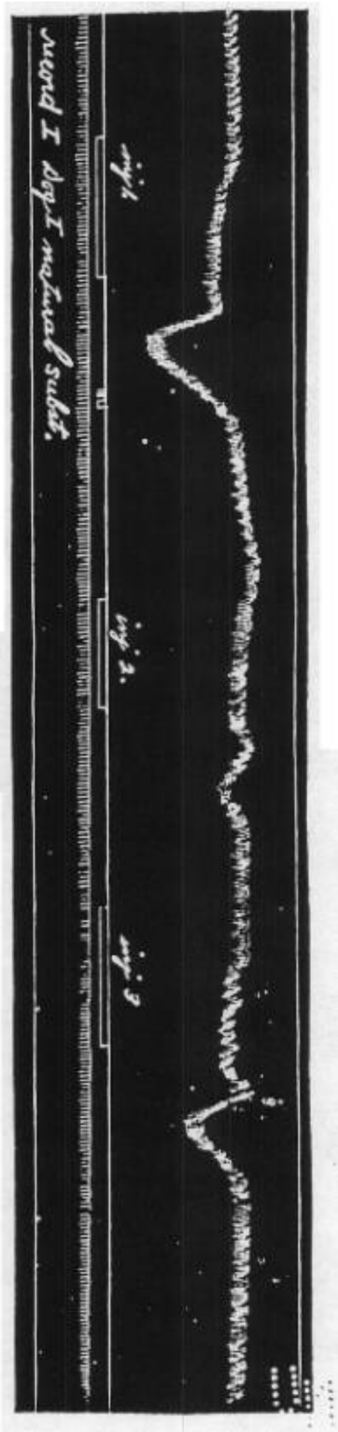
RECORD A-1	
(Dog 1)	
Injections given by jugular vein	
Quantity-5 c.c. 0.6 mgms. of natural substance.	
Fall in pressure from first injection.	about 30 mm. Hg.
Fall in pressure from second injection.	about 5 mm. Hg.
Fall in pressure from third injection.	about 20 mm. Hg.
Heart rate before injecting.	114 per minute.
Heart rate after injecting.	114 per minute.
Interval between injection 1 and injection.	about 72 seconds.
Interval between recovery from first and administration of second injection.	about 36 seconds.
Interval between injection 2 and injection 3.	about 45 seconds.

RECORD A-II.	

(Dog 1)	
Injections given by jugular vein.	
Quantity-5 c.c. 0.6 mgms. of synthetic substance.	
Fall in pressure from first injection	about 25 mm. Hg.
Fall in pressure from second injection.	0 mm. Hg.
Heart rate before injecting.	138 per minute.
Heart rate after injecting.	136 per minute.
Interval between injection 1 and injection 2.	about 60 seconds.
Interval between recovery of first and administration of second injection.	about 30 seconds.



RECORD A-II.



RECORD A-I.

There are then several indications that the tetany of parathyroid insufficiency is due to an intoxication. Namely, that it is subdued by increased diuresis, by the neutralization of toxic basicity, or by the destruction of a toxin by acidity. That the origin of the hypothetical toxic substance is the body itself, that it is useful and not toxic in the presence of the parathyroid glands, and that it is filterable through the glomerulus of the kidney, (and thus, readily diffusible as is shown by the value of diuresis in controlling the tetany) point to a substance "hormone-like" in nature and therefore, very unstable chemically.

It is the object of the present investigation to ascertain the presence and identity of such a substance in the urine of parathyroidectomized dogs and to study its physiological properties. As may be anticipated, the isolation of an unstable substance from the complex urine presents several difficulties, which exclude the hope of quantitative results. My object, therefore, was to isolate

this substance only in sufficient quantities for identification and experimental purposes. After a careful study of the urine, the following method was adopted. Its special advantages depend upon its simplicity, rapidity, and the avoidance of destructive, chemical reagents.

THE METHOD OF ISOLATION

The urine was collected separately from forty-seven parathyroidectomized dogs. Especially designed cages were used to avoid fecal contamination. The urines were filtered and evaporated to a syrup, by an electric fan at a temperature not above 20° C. The residues were dissolved in alcohol, filtered, and evaporated; this process was repeated until the last evaporate dissolved readily in alcohol. The lipoids present were extracted with ether and the residue taken up in water. This solution was cautiously precipitated with picrolonic acid. Several insoluble picrolonates were thus obtained, and by re-crystallization from water and alcohol were purified.

These substances were tested for physiological activity. Two of them were found to modify the blood pressure when injected intravenously into anesthetized dogs. And when injected intra-peritonealy into non-anesthetized animals, they exhibited very marked toxic effects. They were therefore selected for analysis.

One substance was found to reduce gold chloride quite rapidly and picrolonic acid slowly. I therefore tested for an aldehyde group. With ammoniacal silver nitrate no mirror was obtained, but instead a yellow-gray precipitate whose solubility resembles that of silver cyanide. The substance, itself, freed from picrolonic acid is practically neutral in reaction, very soluble in water and alcohol, and somewhat soluble in ether. It gives a picrolonate in the form of very fine microscopic needles, which melt at a 118° C. solidifying rapidly to an orange-colored mass, which melts with decomposition at about 230° C. 0.249 gms. are soluble in 100 c.c. of hot water.

Upon analysis it gives the following percentage composition:

0.1060 gms. substance gives 26 c.c. N. at 24° C. and 746 mm.
0.1783 gms. give 0.2933 gms. CO(2) and 0.0696 gms. H(2)O.

FOUND	CALCULATED FOR
	(C(2)H(4)N(2)) (C(10)H(8)N(4)O(5))
N 26.49	26.31
C 44.86	4.97
H 4.34	3.75

The substance agrees in percentage composition with the picrolonate of Methyl-cyanamide. Methyl-cyanamide was prepared synthetically from methyl mustard oil. It resembles the naturally occurring substance both in reaction and solubilities. Its picrolonate was prepared and found to melt at 116° C. solidifying immediately to an orange-colored mass, which melted at 230° C. with decomposition. In this respect, the picrolonate agrees in its behavior with the picrolonate of the naturally occurring substance.

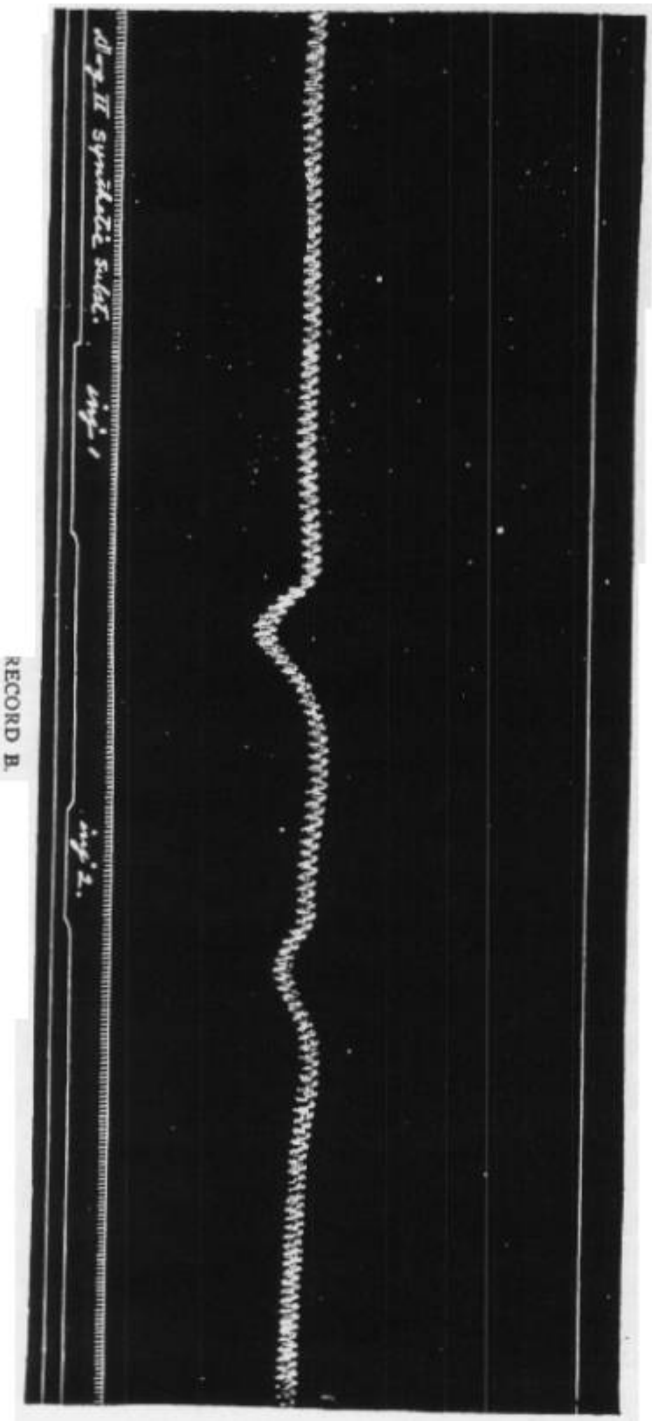
The other substance isolated from the urines and having a physiological action is basic in reaction. It is less soluble in water than the first substance. Its picrolonate crystallizes from water forming small orange-colored mounds. The picrolonate melts at 232° C. with decomposition. It gives on analysis the following percentage composition:

0.1096 gms. give 26.2 c.c. N. at 24° C. and 750.7 mm.
0.1288 gms. give 30.2 c.c. N. at 22.5° C. and 751.3 mm.
0.1731 gms. give 0.2827 gms. CO(2) and 0.063 gms. H(2)O.
0.1345 gms. give 0.0520 gms. H(2)O and 0.2184 gms. CO(2).

FOUND	CALCULATED FOR		
	C(0)H(12)N(6))(3) (C(10)H(8)N(4)O(5))(3)		
	a	b	c
N	26.6	26.28	26.31
C	44.54	44.28	44.97
H	4.07	4.32	3.75

The substance agrees in composition with the picrolonate of Trimethyl-melamine, which is the polymer of Methyl-cyanamide. The polymer was prepared from the synthetic Methyl-cyanamide and found to agree in reaction with the natural substance. The picrolonate of the synthetic polymer was found to melt at 229.4° C. that is slightly below the melting point of the natural salt. This discrepancy can be explained by the presence of small quantities of Methyl-guanidine in the synthetic preparation, which owing to the similarity in solubility of the two picrolonates, could not be completely removed by fractional re-crystallization. It was found that Methyl-cyanamide polymerizes after a few days' standing, or several evaporations of its watery solution. Therefore, the polymer should be expected in the urine from which the cyanamide was isolated.

Because of the agreement in chemical and physiological properties (discussed below) the substances may be considered identified as Methyl-cyanamide and Trimethyl-melamine.



RECORD B.

RECORD B.	
(Dog 2)	
Injection given by femoral vein.	
Quantityâ€”5 c.c. 0.4 mgms. of synthetic substance per kilo body weight.	
Fall in pressure from first injection.	about 25 mm. Hg.
Fall in pressure from second injection.	about 10 mm. Hg.

Interval between injection 1 and injection 2.	about 50 seconds.
Interval between recovery from the first injection and administration of the second	about 20 seconds.

Note: The heart rate cannot be estimated because while taking the tracing, the recording pointer of the chronograph fell off. After the tracing was finished, we attempted to supply a time record, but it did not prove to be synchronous with the former rate of movement of the drum, which was running down. The time record was therefore valueless.

RECORD C.	
(Dog 2)	
Injection given by femoral vein.	
Quantityâ€"5 c.c. 0.4 mgms. of synthetic substance per kilo body weight.	
Fall in pressure from injection 3.	about 25 mm. Hg.
Fall in pressure from injection 4.	about 0 mm. Hg.
Fall in pressure from injection 5.	about 0 mm. Hg.
Heart rate before injecting.	108 per minute.
Heart rate after injecting.	108 per minute.

PHYSIOLOGICAL PROPERTIES

The Methyl-cyanamide, isolated from the urines and the synthetic Methyl-cyanamide, was injected intra-peritonealy in non-anesthetized dogs and found to have similar effects. In small doses they produce extreme vasodilatation observed in the reddening of the sclera, and in the swelling and reddening of the tongue. Larger doses cause paralysis and convulsions. Still larger doses cause an extremely rapid death. Intra-peritoneal injection of 27 mgs. of the synthetic substance into a rabbit of one and one-half kilos body weight produced, after sixteen minutes, very marked vasodilatation observable in the air vessels and in very labored breathing accompanied by wheezing, suggestive of bronchial stricture. In this condition, the animal rapidly developed tremors and tetany with the head thrown back and the hind limbs extended. After a few minutes, the tetany gave way to a coma resulting in death. An injection of 22 mgs. of the natural substance into a rabbit of one and one-quarter kilos body weight produced death, very similarly, although the tetany was not marked and the dyspnea and the coma symptoms predominated. On the other hand, 4 mgs. injected intra-peritonealy into a white rat of about a 100 gms. body weight caused death, practically instantaneously, with opisthotonus developing but no coma whatsoever.

In order to obtain the base for injection, the picrolonate was dissolved in a small quantity of alcohol and decomposed with a calculated quantity of sodium carbonate. The picrolonic acid is thus precipitated as a sodium salt and filtered off. The filtrate was evaporated by an electric fan to dryness and the residue dissolved in water. In this way, a solution of known concentration was obtained. The synthetic substance was prepared fresh each time it was used, and from a known quantity of Methyl-thiourea.

Blood pressures were taken by canulae in the carotid, from dogs anesthetized with chlorotone. For Record A-I, the natural substance, for Record A-II, the synthetic substance was injected into the jugular vein. For Records B and C, the synthetic substance was injected into the femoral vein; experience having shown that injecting into the jugular disturbed the canulae.

Record A-I shows the effect of injection of an aqueous solution containing 0.6 mgms. of substance per kilo body weight. Five cubic centimeters of this solution were given with each injection. The first injection was immediately followed by a sharp drop of about 30 mm. of mercury in the blood pressure. A second injection of the same quantity given fifteen seconds after recovery from the first showed only a slight change; a third injection following the second, by the same interval, had an effect less than the first and more than the second. It appears that the animal had become noticeably refractory to the substance after the first injection, and had recovered from this refractoriness considerably by the time of the third injection. This refractoriness is observed whether the natural or synthetic substance is injected, and its duration toward either substance varies with the animal. In our experience, the older dogs have a longer refractory period than the younger dogs. The dog from which Record A was taken recovered almost completely after 140 seconds; the dog from which Record B was taken required between 80 and 360 seconds for a complete recovery.

To explain this refractoriness and the quantitative relations discussed below, it might be assumed that the Methyl-cyanamide acts in conjunction with some receptor substance slowly elaborated by the body to produce the vasodilatation, and thus the fall in blood pressure. The refractoriness would then be due to the exhaustion of the receptor.

In Record B the first injection did not use up all of the receptor, since another injection followed in about 25 seconds, as a return in the blood pressure to normal, still produced an effect. That the detectable receptor is not generated in this dog within 25 seconds is shown in Record C, where injections 4 and 5 caused practically no pressure change, though given at much longer intervals. It appears, therefore, that injection 4 produced the blood pressure’s fall working with the excess of the receptor not used by injection 3. The above described relations of the cyanamide and the receptor indicates that they react quantitatively to produce the fall in blood pressure.

It is observable in all of the tracings that any excess of the cyanamide, not reacting with the receptor to produce a fall in blood pressure, is nevertheless disposed of quite rapidly, since where sufficient time has elapsed for the generation of noticeable quantities of the receptor, no fall in pressure occurs until further injection of the cyanamide is given. It may be this reaction of the cyanamide in combining with other groups within the organism that produces the tetany and the other symptoms eventually leading to the death of the animal.

It seems that this substance excreted by the parathyroidectomized animal is considerable in quantity. For despite its instability, from the urines of 47 experimental dogs, 1.2 gms. of the cyanamide were isolated and 2 gms. of the polymer, as picrolonates, the quantities actually present in the urines, or the portions decomposed during the isolation, cannot be surmised. Nor would such data be an index to the quantity generated in the organism since during the process of intoxication, a considerable portion would be disposed of.

A comparison of the toxicity of the urines of a dog before and after parathyroidectomy shows that during non-fatal tetany the urine is somewhat toxic, but that after fatal tetany the urine is much more toxic. This is shown by the following experiment: A dog weighing 18 kilos was parathyroidectomized. 8 c.c. of the urine excreted before operation, produced no marked toxic symptoms when injected into a rat of about 100 gms. 5 c.c. of 90 c.c. of urine excreted during a half day, in which the dog was in moderately severe tetany, and upon its injection into another rat of the same size produced mild opisthotonus from which the rat recovered in about ten minutes. The urine, after death, in a quantity of 5 c.c. produced severe and almost fatal tetany upon its injection into a third rat of equal weight. The urines obtained between tetany periods in quantities of 5 c.c. were not toxic. It may be approximated that a dog of 18 kilos, excreting about 200 c.c. of toxic urine, gives off from 10 to 80 mgms. of the cyanamide, if we assume that the 5 c.c. injected contain about 1 to 2 mgms. of the substance since 4 mgms. is toxic to a rat of equal size.

The similarity in the behavior of the parathyroidectomized dogs, to that of the non-anesthetized animals treated with the substance isolated from the urine, is further indication that this substance is responsible for the symptom complex of parathyroid insufficiency. The data, therefore, justifies the following conclusions:

1. Somewhere in the body Methyl-cyanamide is generated.
2. This substance has a physiological value in normal animals.
3. After parathyroid extirpation, the substance accumulates to toxic quantities, and is responsible for the death of these animals.

A further study of some of the problems developed from this investigation is receiving attention.

OPERATIVE AND POST-OPERATIVE PROCEDURES

The operation consists of complete removal of the thyroid and parathyroid glands, together with the surrounding capsular tissue. The essentials of the operative technique are:

1. Thorough asepsis.

2. Prevention of post-operative hemorrhage.
3. The least possible shock and impairment of vitality.

The aseptic methods are those common to most operating rooms and scarcely require detailed description. The same rigid precautions must be observed as in any major operation. The reason for this care is that the oozing of blood and lymph from the rich capillary bed in which the glands are implanted produces ideal media for the development of pyogenic organisms. This is to be avoided not only because the well-being of the animal is reduced by such infection, but also because it is desirable to obviate as far as possible any discharge from the wound. Another feature not to be ignored is the matter of bacterial growth within the wound; it is that the absorption of its products may affect the constituents of the urine. A specially devised mask that covered the muzzle of the animal, but could not slip down on the field of operation was used. The field of operation was shielded from the mask and hands of the anesthetist by a sterile cover. All the loose dirt and hair are removed from the animal's body by a thorough scrubbing with soap and water and a subsequent drenching with warm 1-1000 dichloride of mercury solution. This is done some time prior to the operation.

To prepare the field of operation, the animal's neck is shaved (over the entire anterior aspect) from the angle of the mandible to the juncture of the neck with the thorax. This is best done immediately prior to the operation. After shaving, the skin is scrubbed with soap and water, dried with alcohol, and then painted with iodine.

Operation-The following landmarks must be identified: hyoid bone, sterno-mastoid muscles, larynx, thyroid notch, and trachea. The operator must carefully keep the animal's head directly in line with the rest of the body, or the relationship of the parts will be distorted.

Incision is made from a point exactly at the middle of the inferior border of the hyoid bone to a point in the midline at the third ring of the trachea. The first point may be determined by palpating the notch, between the two thyroid cartilages of the larynx, which locates the midline precisely. Such an incision will be about three inches long. The superficial cervical fascia is then incised by gently drawing the knife over its surface. This exposes the platysma fascia, which is cut in line with the original incision. It is better to cut through these fascias separately as I have described, because occasionally the anterior jugular vein or some of its larger branches cross the line of incision, and these may be clamped before cutting through the platysma fascia. Thus no hemorrhage occurs to obscure the field or cause unnecessary delay. Passing through the platysma fascia or superficial layer of the deep cervical fascia, the infrahyoid muscles overlapped by the sterno-mastoid muscles lie in close proximity on either side of the midline. The point of a four-inch scissors is then inserted in the furrow between the inner borders of the two muscles and the inter-muscular septum severed by spreading the scissors. This septum is more readily identified if care has been taken to make the previous incisions in the midline and the animal's neck has been retained in the anatomical position. The last step brings us down to the surface of the trachea and larynx covered only by the middle layer of the deep cervical fascia. In the dog, the thyroid gland is represented by two lobes, one on either side of the trachea, each lying in a fibrous sheath derived from the middle layer of the deep cervical fascia. As a rule there is no isthmus or pyramidal process as in the human thyroid. There was a marked variation in size of the individual thyroids we examined, as well as in their position, some being immediately below the larynx and others occurring even an inch lower.

One of the lobes is now sought for and brought up into the wound together with the loose fibrous tissue attached to it. It is then seized with a strong hemostatic forceps and light tension is brought to bear on the two poles by which it is fastened. The main branch of the inferior thyroid artery, together with its accompanying vein, is then clamped along with a part of the capsular fascia, which is a loose structure and is readily drawn up. The artery is then ligated proximally to the clamp and the ligature anchored in the fascia. A ligature is also passed around a portion of the remaining fascia in the clamp and this is also anchored, if it includes many vessels. The artery and portion of fascia thus ligated, are then cut between the ligatures and the hemostat. The assistant should hold the stump with tissue forceps, as it is cut and not release it until it is ascertained that there is no bleeding. The fascia remaining attached to the lower portion of the gland is caught up in a hemostatic forceps and fractionally ligated, a small portion being included in each ligature. The importance of rigid hemostasis must not be underrated for it is to be borne in mind that following this operation, there is a marked vascular dilatation all over the body and considerable oozing may take place from vessels, which at the time of operation appear insignificant. If the capsule is carefully ligated in this manner, we are certain to control a great deal of lymphatic seepage. Care must be taken when clamping the capsular tissue that the descending branch of the ansa hypoglossi is not included. If raised, it may be pushed out of the field by gently wiping with dry gauze. The recurrent laryngeal nerve which supplies the intrinsic muscles of the larynx must not be cut as stridulous breathing may occur to say nothing of the discomfort the animal would incur in the loss of his powers of vocalization. It should be identified and pushed out of the field of operation.

Occasionally, small vessels pass into the gland from the sterno-thyroid muscle and these must be securely ligated. After the inferior pole has been freed, it is drawn well up out of the wound. This brings the superior thyroid artery with its branches and the superior thyroid vein into view. The artery gives off a number of small branches close to its origin, the most of which are distributed to the glands, others going to the infrahyoid muscles and larynx. The parathyroid bodies frequently lie in the adventitia of this artery and the surrounding tissue. For these reasons, it is necessary to ligate the artery close to its origin. Occasionally the artery is very short, so that there is scarcely room to insert a hemostat between its origin from the carotid and the gland. These are factors that increase the necessity for making ligatures very secure. The largest branch is first clamped and ligated, the capsule is then clamped a small portion at a time, and the vessels held in the clamp ligated and cut. The ligatures must be drawn tight, but not so tight as to cut through. The operator proceeds in this manner from the lower, outer portion of the capsule about the superior pole of the upper and inner extremity. It is best to secure the larger vessels in a hemostat before cutting. The hemostat is left on the stump until all the vessels have been cut. This can only be practiced where the artery is long enough to permit a long stump. At the upper and inner part of this pole several small vessels from the trachea will be encountered (the interior tracheal vessels which are branches from the inferior laryngeal). They are sometimes overlooked and produce considerable bleeding. The ligatures are all strongly anchored in the fascia. Before releasing the stump, it must be seized with the tissue forceps and carefully sponged and inspected for any oozing. If care has been observed to include every bit of connective tissues at the superior pole between the ligatures, there will be no bleeding. In this manner, the gland is dissected out from below upward and everything is ligated as it is cut. We proceed in the same manner with the gland on the opposite side. After both lobes are removed, the region is carefully inspected for accessory lobes. When any are found they are removed.

The wound is closed by three rows of sutures. The sterno-hyoid muscles are approximated by three interrupted sutures, placed equal distances apart. We used a small curved needle

- DR. KOCH’S EXPLANATION OF THE FUNCTION OF HIS REAGENTS