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Studies on the Chemical Nature of Parathyroid Hormone

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STUDIES ON THE CHEMICAL NATURE OF PARATHYROID HORMONE

by

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Vita

I was born in Chicago, Illinois on June 30, 1911. I attended the Helge A. Haugan elementary school and the Robert A. Waller High School, both in Chicago, and graduated in the summer of 1925 and the spring of 1929, respectively. Following one and a half years of pre-medical training at Crane Junior College in Chicago, I entered the Loyola University School of Medicine in the fall of 1930. I received my Bachelor of Science in Medicine degree in 1932 and served as teaching fellow in Physiological Chemistry during the year 1932-1933.

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INTRODUCTION

The experiments discussed herein are concerned with the chemical nature of the blood calcium-raising principle of the parathyroid gland. Active extracts of the parathyroid gland have been shown by previous workers (1,2) to give most of the common color reactions indicative of protein. In the light of our present knowledge it seems most probable that the hormone is either a protein or a protein derivitive. On this assumption there was ample precedent for carrying out studies in an attempt to determine whether or not certain chemical groups, known to influence the reactions of proteins, are present. According to Gortner (3), some of the groups which may, and probably do occur in the unaltered protein molecule are: the primary amino group ($-NH_2$); the carboxyl group (-COOH), especially in the dicarboxylic acids; the aliphatic alcohol group (-OH); the phenolic hydroxyl group (-OH); an alcohol group intermediate between the aliphatic and the aromatic (-OH), as the hydroxyl group in oxyproline; the imino group (=NH); the acid amide group ($-CO-NH_2$); the sulfhydril group (-SH), as in cysteine or the disulfide group (-S-S-), as in cystine; the alpha hydrogen of tryptophane; and the guanidine nucleus.

Previous work in this laboratory by W. R. Tweedy and M. Torigoe (2) has produced strongly indicative evidence that a nitrogen containing group, either an amino ($-NH_2$), an imino (=NH), or an acid amide group ($-CONH_2$) is essential for the physiological activity of the hormone. Evidence is shown in this paper that parallel with progressive deamination of a standard parathyroid hormone preparation, by nitrous acid there occurs a gradual loss in physiological activity, which may be due to the deaminizing or to the oxidative effect of nitrous acid. Further evidence is offered that a carbonyl group is not present in the hormone molecule and that the hydroxyl group may not form an essential part of the hormone.

The sulfhydril test, performed according to Sullivan's technique (4) indicates that free sulfhydril groups are not present in the hormone molecule. Tweedy and Smullen (5) obtained a negative lead acetate test for sulfur in their purest hormone preparation, from which it appears that neither cystine nor cysteine forms an essential part of the hormone.

METHOD OF BIOLOGICAL ASSAY

The method of biological assay of the hormone has been based upon its known effect of producing a measureable calcium increment in the blood plasma of dogs, which reaches its maximum value several hours after subcutaneous injection. The technique of analysis has been fully

TABLE I

Normal Variation of the Blood Plasma Calcium of Fasting Dogs

No.	Dog	Sex	Weight	Blood Plasma Calcium (Average of duplicate analyses)		
				Initial	After 15 to 16 Hours	Difference
			kgs.	mg. %	mg. %	mg. %
1	05	M	11.6	11.90	12.10	0.20
2	06	M	13.6	12.60	11.80	-0.80
3	07	M	11.1	12.00	12.20	0.20
4	09	M	23.0	12.10	12.60	0.50
5	P2	M	24.4	11.70	12.00	0.30
6	P8	M	13.0	10.57	10.57	0.00
Average.....			16.1	11.81	11.87	0.06

described by M. Torigoe (6). The procedure of determining a dosage of hormone sufficiently large to produce an increase in the blood calcium of any dog, has been arrived at in a slightly different way than that reported above (6).

A series of tests on dogs (TableI) of both sexes, and of various sizes, kept under the same environmental conditions as other animals used in subsequent potency tests, shows that the normal variation in the blood plasma calcium may not exceed 0.65 milligrams per 100 cc. in the course of 15 hours.

A second series of animals (Table II) which received one milligram of the hormone preparation per kilogram body weight did not uniformly show a blood plasma calcium increment at the end of 15 hours.

Dogs 5 and 2 (Table II) showed a positive calcium increment of only 0.05 and 0.90 milligrams, respectively, at the end of a 15 hour period. The first value falls within normal variation and the second is only slightly indicative of hormone effect. However, the average blood plasma calcium increment for the entire group of ten dogs was 1.73 milligrams. From these results it appeared that a dose of three milligrams per kilogram body weight should produce in the average dog a blood plasma calcium increment of 5.19 milligrams, 15 hours after subcutaneous ad-

TABLE II

Blood Plasma Calcium Increment produced by Administration of Parathyroid Hormone

No.	Dog	Sex	Dose	Weight	Blood Plasma Calcium (Average of duplicate analyses)		
					Initial	After 15 to 16 Hours	Difference
			mg.	kgs.	mg. %	mg.%	mg. %
1	M9	M	22.7	22.7	12.55	14.55	2.00
2	03	M	11.3	11.3	11.60	12.50	0.90
3	04	M	34.8	34.8	11.85	13.20	1.35
4	C	M	14.5	14.5	13.30	15.00	1.70
5	A	M	20.0	20.0	12.20	12.25	0.05
6	G7	M	22.9	22.9	12.50	13.65	1.15
7	09	M	23.0	23.0	11.85	14.70	2.85
8	P3	M	14.1	14.1	11.30	13.90	2.60
9	P4	M	11.6	11.6	11.35	12.80	1.45
10	P5	M	21.7	21.7	11.70	14.90	3.20
Average....			19.7	19.7	12.02	13.75	1.73

ministration. Since it was also desirable to fix the size of dosage necessary to accomplish this, a larger series consisting of twenty dogs was used (Table III).

TABLE III

Blood Plasma Calcium Increment produced by Administration of Parathyroid Hormone

No.	Dog	Sex	Weight	Dose	Blood Plasma Calcium (Average of duplicate analyses) Initial	After 15 to 16 Hours	Difference
			kgs.	mg.	mg. %	mg.%	mg.%
1	A1	M	15.0	42.0	11.89	16.67	4.78
2	A4	F	28.6	89.0	11.48	18.56	7.08
3	A6	M	24.7	61.8	11.51	17.37	5.86
4	B3	F	16.8	50.0	11.51	13.21	1.70
5	B5	F	17.3	52.0	11.30	19.35	8.05
6	B8	F	15.5	46.0	11.30	14.89	3.59
7	C1	M	14.7	44.0	11.03	14.44	3.41
8	C2	M	14.7	44.0	13.65	21.13	7.48
9	C4	F	13.9	42.0	13.00	19.00	6.00
10	C6	M	13.2	39.5	13.10	15.79	2.69
11	A6	M	25.9	77.7	11.80	16.54	4.74
12	D1	M	21.5	64.5	12.23	16.62	4.39
13	D2	M	13.1	39.3	11.80	15.01	3.21
14	D3	M	17.5	52.5	11.25	14.36	3.11
15	D5	M	12.2	37.0	10.62	16.05	5.43
16	D6	F	28.2	84.6	10.98	14.10	3.12
17	D1	M	21.7	64.8	10.07	17.07	7.00

TABLE III (Continued)

No.	Dog	Sex	Weight kgs.	Dose mg.	Initial Calcium mg. %	After 15 to 16 Hours mg. %	Difference mg. %
18	D7	M	24.3	72.9	11.51	17.58	6.07
19	D8	M	25.0	75.0	9.92	14.37	4.45
20	D9	M	15.0	45.0	11.84	14.45	2.61
Average....			18.99	56.1	11.59	16.33	4.74

Smallest Calcium increment.....1.70

Largest Calcium increment.....8.05

In table III it will be observed that the lowest calcium increment obtained for a dose of 3 milligrams per kilogram body weight was 1.70 milligrams, while the highest value was 8.05 milligrams (No. 4 and 5, respectively). The average increase in plasma calcium for the entire group of 20 dogs was 4.74 milligrams, a difference of only 0.45 milligrams from the value calculated from the data in table II.

Therefore, it may be concluded that while there is a wide divergence in response to comparable doses of the hormone, its average calcium increasing effect can be calculated with a reasonable degree of accuracy providing that a sufficiently large number of animals are used in

potency testing. The data recorded here confirm Collip's claims (7) in regard to the reliability of his method of parathyroid hormone standardization.

EXPERIMENTAL

1. Effect of phenylhydrazine on the physiological activity of parathyroid hormone.

It was assumed that if parathyroid hormone contained a carbonyl group, it should be inactivated by contact with phenylhydrazine, since this reagent is known to react with the carbonyl group. In the first experiments* pure freshly redistilled phenylhydrazine was used. This was placed in contact with parathyroid hormone in a tube of appropriate size, and the air was displaced by oxygen-free nitrogen. It was judged that all oxygen was out of the tube when the escaping gas failed to produce a precipitate of manganic oxide when bubbled through an aquaeous solution of manganeous sulphate. The stoppered tube was allowed to stand at room temperature (approximately 22°C) for a period of twenty-four hours. The contents of the tube

*Unpublished experiments by W. R. Tweedy which are described here with his permission.

were then mixed with ether, centrifuged, washed with ether several times, and dried. Tweedy found that phenylhydrazine used under the above conditions produced no inactivation of the hormone. These experiments were repeated, the only variations being that the commercial, instead of freshly redistilled phenylhydrazine was used, and air was not excluded. This was done in order to establish the importance of the precautions observed in the first experiments.

TABLE IV

Effect of Phenylhydrazine on the Physiological Activity of Parathyroid Hormone

No.	Reagents added to the phenylhydrazine	Time Hrs.	Temperature °C	Calcium Increment mg. %	Dose mg. per kg.
1	Absolute CH CH	24	20	6.79	3.00
2	Absolute CH CH	20	23	1.05	3.00
3	1% Acetic acid	0.5	24	2.78	3.00
Average.....		14.8	22.3	3.54	3.00

Only three experiments were performed. The first two were carried out under similar conditions, and although there is a large difference in degree of hypercalcemia

produced in the test animals, the values fall within the range of animall variation and indicate that the hormone is little if any affected by the above treatment. These experiments taken in conjunction with the experiments of W. R. Tweedy indicate the absence of a carbonyl group in the hormone molecule.

2. Effect on the stability of parathyroid hormone of alkali of various concentrations in relation to time and temperature.

Very little information has previously been obtained on the stability of the parathyroid hormone to alkali. Collip (1) states that parathyroid hormone is inactivated by boiling in 5% sodium hydroxide for one hour. Later, Allardyce (8) states that parathyroid hormone extracts made just alkaline to phenolphthalein are not inactivated on standing for four hours at room temperature. Tweedy and Torigoe (2) state that parathyroid hormone is uninjured by contact with 0.08 N alkali for thirty hours at ice box temperature.

More detailed information was desirable in order to determine the use that might be made of alkali in reactivation (deacetylation) of parathyroid hormone inactivated by acetic anhydride, the assumption being that inactivation by the latter reagent may have been due to acetyl-

TABLE V

Effect on the Stability of Parathyroid Hormone of Alkali of Various Concentrations in Relation to Time and Temperature.

No.	Concentration of Alkali	Time of Contact	Temperature	No. of Dogs	Plasma Calcium Increment		
					Lowest	Highest	Average
		Hrs.	°C		mg. %	mg. %	mg. %
1	N/100	17	0-2	1	--	--	5.79
2	N/30	24	0-2	2	3.13	7.84	5.84
3	N/30	66	0-2	1	--	--	5.71
4	N/30	246	0-2	1	--	--	1.61
5	N/100	6	37-38	1	--	--	5.16
6	N/30	2	37-38	1	--	--	6.22
7	N/30	23	37-38	1	--	--	0.40
*8	N/20	5	37-38	14	0.20	3.94	1.73
9	N/17.4	5	37-38	2	0.95	5.46	3.21

*This group consisted of 11 separate experiments.

ation of hydroxyl groups. Secondly, it was thought that such information might be useful in either the preparation of parathyroid extracts or in subsequent studies on the effects of other reagents involving the use of alkali.

From the data shown in table V, it is evident that the physiological activity of the hormone is unaffected by contact with either N/100 alkali for 17 hours at 0-2°C or with

N/30 alkali for a period of time as long as 66 hours. However, under the latter conditions of alkali concentration and temperature, if the time is extended to 246 hours, loss of activity is indicated.

A series of experiments were then performed in which the severity of treatment was increased by raising the temperature for the period of contact to 37°C. It will be noted, however, (Table V) that contact with N/100 alkali for 6 hours, or N/30 alkali for 2 hours at 37°C was without effect. In the latter case when the period of contact was increased to 23 hours, definite loss in activity was indicated.

It was then decided to make a more quantitative estimation of the loss in physiological activity produced by contact of the hormone with alkali of definite strength at a constant temperature for a stated period of time. In this series of experiments, contact with N/20 alkali was maintained for 5 hours at 37-38°C in a closed tube. Immediately after the incubation, as in previous experiments, the solution was cooled to room temperature and the pH adjusted to between 4.8 and 5.2 preliminary to injection into the test animal. A series of fourteen biological potency tests ere then made (Table V, No. 8). The average calcium increment produced for a three milligram per kilogram dose was found to be 1.73 milligrams. As is shown in

table II, the above value corresponds to the calcium increasing effect produced by the injection of a one milligram per kilogram dose of the original hormone. The data indicate with a reasonable degree of accuracy that under the above conditions of incubation, approximately two thirds of the original activity has been destoryed. The reason for a loss in physiological activity is not known. On the assumption that the hormone is of protein nature, it was thought that the most likely change produced under the given conditions of alkali treatment would be expected to be racemization. Accordingly, polariscopic examination was made immediately after contact with N/20 alkali and after 5 hours incubation at 37-38° C. Due to the brown colored impurity associated with the preparation, which cannot be removed by shaking with activated charcoal, considerable difficulty was experienced in obtaining close checks on the polariscopic readings. However, it was observed that no significant change in optical rotation was produced under the above conditions of alkali treatment.

3. Effect of acetic anhydride on the physiological activity of the parathyroid hormone in relation to time and temperature.

A series of preliminary experiments have been carried out to determine whether or not the physiological activity

TABLE VI

Effect of Acetic Anhydride on the Physiological Activity of Parathyroid Hormone in Relation to Time and Temperature

No.	Reagent	Time Hrs.	Temperature °C	No. of Dogs	Dose mg./kg.	Actual Calcium Increment mg. %	Calculated Calcium Increment mg. %
1	Pyridine Acetic Anhydride	0.75	0	2	4.5	2.09	1.39
2	"	3.00	0	1	6.0	0.90	0.45
3	"	3.00	0	1	6.0	3.56	1.78
4	"	53.00	0	1	5.0	2.13	1.28
Average		14.94	0		5.4	2.17	1.23
5	Acetic Anhydride "	20.0	0	2	3.00	1.59	1.59
6	"	20.0	0	2	3.00	4.58	4.58
Average		20			3.00	3.09	3.09
7	"	2.0	R.T.	1	2.8	2.10	2.25
8	"	4.0	R.T.	1	3.78	3.73	2.96
Average		3.0			3.29	2.91	2.61
9	"	1.0	37	1	4.5	3.98	2.65
10	"	1.5	37	1	4.1	1.05	0.77
11	"	2.0	37	1	4.0	0.41	0.30
12	"	2.0	37	1	4.5	2.27	1.51
13	"	2.5	37	1	4.1	1.76	1.26
14	"	3.0	37	1	4.6	1.96	1.28
Average		2.0			4.3	1.91	1.30

of the hormone is affected by treatment with acetic anhydride. The first six of these experiments (Table VI) were carried out under conditions which would not be expected to produce racemization of protein, but which should be favorable to produce inactivation by acetylation at OH, NH_2 , or at =NH groups, if such groups form an essential part of the hormone molecule. In the preparation of the acetylated products (No. 1 to 6, Table VI) the temperature was kept at approximately 0°C, but the time of contact was varied from 45 minutes to 53 hours. The hormone preparation was treated with either acetic anhydride or acetic anhydride in the presence of pyridine, in the ratio of 1 to 2, as will be seen from the table. It appears as indicated in the first four results (Table VI) that partial inactivation of the parathyroid hormone by acetic anhydride in the presence of pyridine occurs within a few minutes. This observation is in agreement with a longer series of unpublished experiments performed by M. Torigoe. In subsequent experiments with acetic anhydride, the use of pyridine was discontinued in the belief that the slower inactivation produced by the acetic anhydride alone might produce a product more easily reactivated by deacetylation methods.

The experiments, numbers 1 to 4, table VI, indicate that inactivation to the extent of 65.2% is produced by

contact with acetic anhydride for 15 hours at 0°C. Approximately the same degree of inactivation was produced (No. 9 to 14, Table VI) by treatment with the acetic anhydride for two hours at 37°C.

Although the conditions in these experiments for the inactivation vary from mild to very drastic treatment, the hormone preparation loses only about two thirds of its activity. Since the remaining one third of the hormone activity is not affected under the most drastic treatment used, it may be that we are dealing with a heterogeneous mixture in which this portion of the hormone is well protected from interaction with acetic anhydride. The experiments on the stability of the hormone to alkali also indicate that under conditions where two thirds of the activity is destroyed, onethird remains intact (No. 8, Table V). Of course, there are otherpossible explanations for the apparent failure to produce complete inactivation. The completely acetylated parathyroid hormone may itself be slightly reactive, or it may be that the acetylated hormone is completely inactive, but after injection is partially deacetylated in the animal body and then exerts its usual effect.

A few experiments have been performed in an effort to reactivate acetylated products. No demonstrable reactivation has been attained, however, (TableVll). In view

TABLE VII

Effect of Alkali on the Physiological Activity of Acetic Anhydride Treated Material

No.	Strength of Alkali	Time Hrs.	Temperature °C	Alkali Treated acetylated material		Acetylated Material	
				Dose mg/kg	Increment mg; %	Dose mg/kg	Increment. mg. %
1	N/30	24	0	3.0	1.52	4.5	3.02
2	N/30	66	0	2.92	0.24	-	--
3	N/100	22	0	4.3	2.62	4.6	1.96
4	N/100	24	0	4.5	2.57	4.5	2.27

of the more detailed information on the stability of the hormone to alkali it will be possible to carry out further experiments.

4. Effect of glacial acetic acid on parathyroid hormone extract.

In subsequent experiments on the effect of small concentrations of nitrous acid on parathyroid hormone, information on the stability of the hormone to glacial acetic acid was desirable. As shown in table VIII, the hormone is very stable to glacial acetic acid. It will be noticed (Table VIII) that the lowest calcium increment obtained af-

TABLE VIII

Effect of Glacial Acetic Acid on Parathyroid Hormone Activity

No.	Mg. of Hormone used	Glacial Acetic Acid cc.	% Recovery	Time Hrs.	Temperature °C	Dose mg/kg	Blood Plasma Calcium Increment mg. %
1	100	10	87.6	5. min	R. T.	3.0	5.95
2	150	15	90.5	1.0	R.T.	3.0	8.05
3	50	5	85.2	2.5	R.T.	3.07	8.59
4	150	15	81.5	8.75	R.T.	3.0	4.65
5	150	15	91.0	11.5	R.T.	3.0	4.95
6	150	15	87.0	100	R.T.	3.0	4.47
7	300	30	75.5	1.0	R.T.	3.0	4.50
8.	-	-	-	-	--	3.0	4.25
*9	-	-	--	1.0	37	3.0	3.85
10	100	10	--	1.0	R.T.	3.0	3.35
11	150	18	--	24.0	R.T.	3.0	4.05
Average.....							5.15

*Material No. 7 treated further for one hour at 37°C.

ter treatment with glacial acetic acid was 3.35, while the highest was 8.59 milligrams. The average increment was 5.15 milligrams. This 0.41 milligram more than that shown for the potency of a comparable amount of the

untreated preparation (Table III). It is also notable that nine out of the eleven animals showed an increase in the blood plasma calcium of 4 to 8.58 milligrams, while only twelve out of twenty showed a corresponding response in the injection of the untreated hormone preparation (Table LLI), and if due allowance is made for the difference in the number of animals tested, there still appears to be positive evidence of an increase in potency by the glacial acetic acid treatment.

The alpha amino nitrogen values obtained for several such preparations were only slightly greater than that of the original preparation, so it is doubtful that this apparent increase in potency is produced by a hydrolytic effict of the acid.

5. Effect of nitrous acid on parathyroid hormone suspended in glacial acetic acid.

In previous work (2) in this laboratory it was found that when parathyroid hormone preparations are completely freed of alpha amino nitrogen, no evidence of physiological activity could be obtained by the ordinary method of testing. It was recognized by these workere (2) that inactivation of the hormone might have resulted by any one of several effects produced by nitrous acid. It was stated that the loss in hormone activity may have been produced

by deamination, oxidation, nitrosation, or possibly nitration. No attempt was made to separately produce these reactions.

The work below was done in the hope that a clearer conception of the inactivating effect of nitrous acid might be obtained if the hormone preparation was subjected to extremely dilute concentrations of nitrous acid. It was hoped that if the loss in potency was the result of the oxidative action, rather than the deaminizing action of nitrous acid, it might become more evident.

In the experiments recorded below, it will be observed that when there is an average loss in alpha amino nitrogen of 28%, the loss in physiological activity may reach 80% (Table IX). From these experiments as well as those recorded in tables X and XI it is evident that the loss in physiological activity seem to be related to only a small portion of the total alpha amino nitrogen. The results recorded suggest several possibilities. 1. It is possible that we are dealing with a heterogeneous mixture in which the hormone is only a small component and that the rate of loss of alpha amino nitrogen from the hormone is more rapid than from other alpha amino nitrogen containing components of the mixture. 2. It may be that we are dealing with two reactions, namely, an oxidative reaction and a deamination reaction (Table XI), which parallel each

TABLE IX

Relation of Calcium Increment to Time of Exposure to Nitrous Acid. Temperature constant at 20-23°C

No.	Time	Mg. used for Van Slyke	Mg. of Nitrogen	Percentage of NH Nitrogen. Lost	Retained	Calcium Increment mg. %
1	5 min	30	.2182	17.6	82.4	4.6
2	5 "	--	--	--	---	2.9
3	5"	--	--	--	--	2.71
Average for 5 minutes.....				17.6	82.4	3.40
4	10	30	.2063	22.2	77.8	2.35
5	30	42.1	.3292	11.3	88.7	2.70
6	40	30	.1728	34.8	65.2	2.15
7	1 Hr.	--	--	--	--	0.23
8	1 "	20	.1371	22.3	77.7	-0.40
9	1 "	57	..3762	25.3	74.7	1.10
10	1 "	54.3	.3315	30.8	69.2	2.17
11	1 "	48.3	.2793	34.6	65.4	0.00
12	1 "	--	--	--	--	2.10
Average for 1 hour.....				28.2	71.8	1.00
13	2 Hr.	49.2	.2636	39.3	60.7	0.05
14	2"	57.8	.3300	35.3	64.7	-1.25
Average for 2 hours.....				37.3	62.7	-0.60

TABLE X

Relation of Calcium Increment to Time of Exposure to Nitrous Acid with Temperature Constant at 26-27°C.

No.	‡Ratio of Reagents	Time Hrs.	Mg. used for Van Slyke	Mg. of Amino Nitrogen	% of Amino Nitrogen* Lost	% of Amino Nitrogen* Retained.	Calcium Increment mg.	Calcium Increment %
1	16:2	0.5	42.1	.3292	11.3	88.7	2.70	
2	16:2	1.0	57.0	.3762	25.3	74.7	1.10	
3	16:2	1.5	46.1	.2575	36.8	63.2	0.10	
4	16:2	2.0	49.2	.2636	39.3	60.7	0.05	
5	17:1	2.0	57.8	.3300	35.3	64.7	-1.25	
6	17.5: .5	1.0	48.3	.2793	34.6	65.4	0.00	
7	17.25: .25	1.0	54.3	.3315	30.8	69.2	2.17	
8	17.97:.03	1.0	--	--	--	--	2.10	

‡ The ratio of reagents expresses the volume of glacial acetic acid to the volume of isoamylnitrite.

* The percentage of alpha amino nitrogen is based on 0.0084 mg. of nitrogen per milligram of material as 100%. This number was determined as the average of two Van Slyke determinations made on the original hormone.

other, but are different in velocity, and that the loss in physiological activity is a result of the oxidative reaction. As shown in the last part of this paper, the ex-

treme sensitivity of the hormone to hydrogen peroxide suggests this to be the most likely explanation.

TABLE XI

Relation of Calcium increment to Percentage of Amino Nitrogen Retained by Partially Deaminized Hormone Preparations

No.	%of Alpha Amino Nitrogen Retained	Dose $\frac{mg}{kg}$	Calcium Increment mg. %	Average Calcium Increment. mg. %
1	88.7	3.00	2.70	
2	82.4	3.00	4.60	
3	77.8	3.00	2.35	3.22
4	77.7	3.00	- 0.40	
5	74.7	3.000	1.10	
6	69.2	3.00	2.17	1.09
7	69.0	3.24	0.45	
8	65.4	3.00	0.00	
9	65.2	3.00	2.15	0.86
10	64.7	3.00	- 1.25	
11	63.2	3.00	0.10	
12	60.7	3.00	0.05	0.05
13	55.4	3.00	0.10	

TABLE XI (Continued)

No.	% of Alpha Amino Nitrogen Retained	Dose mg./kg.	Calcium Increment mg. %	Average Calcium Increment mg. %
14	40.4	3.00	-0.08	
15	37.6	3.00	0.60	0.10
16	35.0	3.00	0.30	
17	31.6	3.00	1.80	
18	--	3.00	0.00	
19	--	3.00	0.60	0.65
20	25.8	3.00	0.15	0.15

The average calcium increment is the average of every three or four dogs.

5. Effect of hydrogen peroxide on the physiological activity of the parathyroid hormone.

The experiments on the effect of nitrous acid in small concentrations suggested that its inactivating effect on parathyroid hormone was due to oxidation rather than deamination. Accordingly more exact information was desired on the stability of the hormone to known concentrations of an oxidizing agent.

The experiments with hydrogen peroxide (Table XII)

show that the hormone is extremely sensitive to the reagent in small concentrations. The mechanism of its action is not understood, but it is suggested that a peroxide may be formed with some component of the preparation which in turn acts as the oxidizing agent. The experiments strongly suggest that inactivation by nitrous acid may most probably have resulted from oxidation rather than deamination.

TABLE XLI

Effect of Hydrogen Peroxide on Parathyroid Hormone

No.	% og Peroxide by Volume	Time Hrs.	Temperature °C	Dose mg./kg.	Blood Plasma Calcium Increment mg. %
1	5.0	5	R.T.	4.04	0.40
2	0.7	1	R.T.	3.00	0.20
3	0.7	1	R.T.	3.00	0.21
4	0.35	1	R.T.	3.00	0.01
5	0.35	1	R.T.	3.00	0.30
6	0.17	1	R.T.	3.00	0.67
7	0.17	1	R.T.	3.00	1.51
8	0.085	1	R.T.	3.00	0.50
9	0.085	1	R.T.	3.00	5.05
10	0.07	1	R.T.	3.00	6.20
11					3.92

The peroxide was titrated against N;2 potassium permanganate.

SUMMARY

1. Experiments with phenylhydrazine indicate that a carbonyl group is not present in the hormone molecule.

2. The hormone has been shown to be very stable to alkali. After the hormone has stood in contact with N/20 alkali for five hours at 37°C there is still about one-third of the original activity present.

3. The conditions for obtaining a certain amount of inactivation of parathyroid hormone by acetic anhydride have been determined. These conditions appear to be more drastic than should be necessary, if inactivation is due to acetylation of a hydroxyl group. No success was obtained in reactivation of such products by treatment with alkali in concentrations known to be harmless to the original hormone. The presence of a hydroxyl group in the hormone molecule is doubtful.

4. The activity of the parathyroid hormone is not decreased after contact with glacial acetic acid for several hours at room temperature. The data are indicative of some increase in potency.

5. From the data obtained on the effect of dilute solutions of nitrous acid on the parathyroid hormone, it appears most probable that inactivation is produced by ox-

idation rather than by deamination.

6. The physiological activity of the parathyroid hormone has been shown to be extremely sensitive to small concentrations of hydrogen peroxide. These data lend support to the above theory of action of nitrous acid on parathyroid hormone.

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