XV. THE NUCLEOTIDE COMPONENT OF MAMMALIAN CARDIAC MUSCLE.

I. THE COMPOSITION AND CHEMICAL PROPERTIES OF THE NUCLEOTIDE.

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II. THE BIOLOGICAL AND CO-FERMENT PROPERTIES OF THE CARDIAC DINUCLEOTIDE.

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I. THE COMPOSITION AND CHEMICAL PROPERTIES OF THE NUCLEOTIDE.

THE presence of an adenosinetriphosphoric acid in mammalian skeletal muscle was discovered independently by Fiske and Subbarow [1929] and Lohmann [1929]. Barrenscheen and Filz [1932] have recently suggested that the labile phosphoryl linkages in this nucleotide are at least in part with the NH_2 of the adenine, their opinion being based mainly upon the difficulties associated with the deamination without dephosphorylation (of the loosely bound phosphorus), whether this is brought about by chemical or by enzymic processes. From an examination of their acid hydrolysis experiments it is evident that the nucleotide which they had prepared from skeletal muscle behaved differently from pure adenosinetriphosphoric acid for, even after 1–3 hours' treatment with N HCl at 100° , only 80–85 % of the loosely bound phosphoric acid was set free, while the same compound prepared by others readily gives off completely two of the three phosphoric acid molecules within ten minutes under the above conditions. From a study of the acid hydrolysis curves of nucleotides certain important differences in the nature of the linkages in purine nucleotides of different origin may be readily observed. Even in the cases of the adenosinemonophosphoric acids of yeast and muscle, it is evident that there is a characteristic difference in the firmness of the single phosphoryl linkage with an alcohol group of the pentose. Thus the phosphoryl linkage of the yeast derivative can be readily broken by acid hydrolysis, while those of the muscle compound and its deaminated derivative, inosinic acid, offer a much greater resistance [Levene and Jorpes, 1929]. The position of the phosphoric acid linkage in the muscle derivatives has been definitely established, namely to the terminal alcohol group of the ribose (C_5), while that of the similar linkage in the adenylic acid of yeast is probably the same as in guanylic acid [Levene and Harris, 1932] namely to C_3 of the ribose. The lability of the phosphoryl linkage in the yeast derivative is of much the same order as that of the two loosely bound

phosphoryl groups in adenosinetriphosphoric acid (a.t.p.) of skeletal muscle. The ease with which the labile linkages in the latter may be broken is shown in Embden's method of preparing adenylic acid from muscle, when under the action of $\text{Ca}(\text{OH})_2$ at room temperature the original molecule loses two of the three phosphoric acid molecules. As will be seen later the nucleotide of cardiac muscle shows certain characteristic differences from that of skeletal muscle, due to the number and possibly also the position of the phosphoryl linkages. It is necessary always to bear in mind that the methods employed in separating the salts of these nucleotides may give rise to alterations in the character of the linkages. Fischer [1914] in his investigation of synthetic theophylline glucoside-phosphoric acid referred to the ease with which the phosphoryl linkage might be altered with reaction, thus the original monobasic acid with two of the OH groups of the phosphoric acid fixed to alcohol groups of the glucose might readily be changed to a dibasic acid with one only of the OH groups fixed to a single alcohol group.

In this communication special attention will be directed to the adenylic nucleotide in cardiac muscle, but it will be necessary to compare it with the closely allied substance met with in skeletal muscle. There are only two definite references, both very brief, to the adenylic derivative of heart muscle. One of these is by Lohmann [1931] in which the following characteristics of this body are just mentioned. The barium salt on neutral hydrolysis was found to furnish adenylic acid and pyrophosphoric acid, and the ratio of the loosely bound to the firmly bound phosphorus was found to be 1 : 1, instead of 2 : 1. The second reference occurs in a paper by Deuticke [1932] dealing with the action of the heart nucleotide on the uterus of the guinea-pig, in which he briefly mentions a statement made by Embden at a congress in Wiesbaden in 1932 to the effect that the substance is probably a dinucleotide consisting of one molecule of adenosinetriphosphoric acid and one of adenosinediphosphoric acid in which the ratio of N : P is therefore 10 : 5. Ostern and Mann [1933], dealing with the deamination process in heart-muscle, refer briefly to the statements made by Lohmann and Embden, but they did not themselves analyse the compound.

Evidence may be obtained very readily of the presence in heart-muscle of a substance possessing approximately two labile H_3PO_4 molecules to one firmly bound. Thus in the trichloroacetic acid extract of the muscle without separation of the substance, as also in the calcium hydroxide precipitate and in the mercuric acetate precipitate, the existence of such a substance is easily shown. When, however, further purification of the nucleotide is carried out in the way to be described, certain differences between the skeletal and cardiac derivatives become evident. The method of separation of the silver salt follows, generally speaking, the procedure originally adopted by Fiske and Subbarow for the skeletal muscle derivative, but their description is very brief.

Preparation of the silver salt of the cardiac nucleotide.

The same salt may be obtained from the hearts of the rabbit, cat, dog and ox, but in order to obtain larger quantities it is advisable to use the heart of the horse. The heart was removed immediately after death, the cavities being slit open and washed out with normal saline solution. The whole organ while still showing fibrillary contractions was placed in a large basin surrounded by freezing mixture and immediately taken to the laboratory for extraction. The more muscular parts were removed and minced. In this way about one kg. of muscle could be obtained, which was then extracted with two volumes of 5 % trichloroacetic acid, filtered, neutralised and again filtered. About two litres of

protein-free extract were obtained. Acetic acid was added up to 2 % and then saturated mercuric acetate solution in 2 % acetic acid until precipitation was complete. The deposit was separated by centrifuge and after washing with weak mercuric acetate solution the mercury was removed by H_2S and the latter by aeration. This solution was found to contain per g. muscle; total P, 0.029 mg.; P as orthophosphoric acid, none; P as orthophosphoric acid after hydrolysis for 10 mins. with N HCl at 100° , 0.02 mg. The solution was then concentrated *in vacuo* at 40° to a volume of approximately 150 ml. To this 20–30 ml. 20 % $CaCl_2$ and ethyl alcohol slightly acidified with HCl were added until precipitation was complete. The precipitate, after repeated solution and reprecipitation, was finally washed free from chlorine by 50 %, 70 % and finally absolute alcohol. After drying, the salt was dissolved in weak nitric acid and 10 % $AgNO_3$ added until the separation of the crystalline salt was complete. The precipitate was thoroughly washed with weaker alcohols and then with ethyl alcohol and ether and finally dried *in vacuo* at 80° over P_2O_5 .

The yields from heart muscle were always much less than from skeletal muscle. The silver salt of the nucleotide in skeletal muscle was prepared in the same way.

Attention was directed especially to the estimation of the total nitrogen and phosphorus, the ribose and the silver.

Methods of analysis.

Nitrogen; by micro-Kjeldahl.

Phosphorus; by Fiske and Subbarow's method.

Ribose; the method employed was to determine the reducing value by Hagedorn and Jensen's method after half an hour's hydrolysis at 100° with N HCl to break the glucosidic linkage. Levene in his determinations of the pentose in the purine nucleotides found that the maximum reducing power was reached after about half an hour's acid hydrolysis and that the yield reached about 96 % of the theoretical value. He determined the reducing value of pure *d*-ribose and found it to be (by Hagedorn and Jensen's method) 87.5 % of the glucose value. Our calculations of the ribose value are based upon these data given by Levene. The furfuraldehyde yield was also determined and was always found to be higher using the method of Andrews and Milroy [1933] than with the Hoffman method.

Silver was estimated by the usual thiocyanate method. In all cases the silver salts analysed were free from inorganic phosphate. As will be seen the analyses of the silver salt of the skeletal muscle derivative correspond closely to a salt with the formula $C_{10}H_{13}O_{13}N_5P_3Ag_3$.

From Lohmann's electrotitration curves [1932] the acid appears to possess in its unbroken form four acid valencies, three primary and one secondary. In the silver salt two of the three silver atoms seem to be taken up by the primary acid groups in the labile diphosphoryl linkage and the other by the primary acid OH of the stable phosphoric acid linked to the terminal alcohol group in the ribose. If the acid be regarded, as appears probable, as tetrabasic with a labile anhydro-diphosphoryl linkage, the above formula includes one molecule of water.

	Calculated for $C_{10}H_{13}O_{13}N_5P_3Ag_3$ (827.84) %	Found	
		Spec. 1 %	Spec. 2 %
N	8.46	8.62	8.29
P	11.24	11.18	11.08
Ribose	18.12	18.00	17.78
Silver	39.09	39.12	38.85

The above are the analyses of the silver salts of two specimens of the skeletal derivative obtained from different sources.

The salt therefore corresponds to one with $N : P = 5 : 3$ and the agreement with the calculated values for silver and ribose is fairly close.

When the analyses of the cardiac derivative are examined such an agreement with an adenosinetriphosphate is no longer evident. For comparison with other possible nucleotide forms, the values found are to be compared not only with the silver salt of adenosinetriphosphate (I), but also with the theoretical values for a corresponding adenosinediphosphate (II) and for a dinucleotide consisting of an equimolecular mixture of these two (III).

	Calculated for			Found	
	I $C_{10}H_{13}O_{13}N_5P_3Ag_3$ (827.84) %	II $C_{10}H_{13}O_{10}N_5P_2Ag_2$ (640.94) %	III $C_{20}H_{26}O_{22}N_{10}P_5Ag_5$ (1468.78) %	Spec. 1 %	Spec. 2 %
N	8.46	10.92	9.53	9.60	9.51
P	11.24	9.67	10.56	9.92	10.00
Ribose	18.12	23.40	20.43	19.80	19.50
Silver	39.09	33.66	36.72	34.82	35.40

Specimens I and II have $N : P = 10 : 4.7$ in place of $10 : 5$ and the silver values are below the theoretical for such a dinucleotide. The salts are however much more closely allied to a mixture of one molecule of adenosinetriphosphate with one of the diphosphate than either of these in the pure form. That the labile phosphoryl linkages in the cardiac muscle derivative differ slightly from those in that of the skeletal muscle is evident from the results of hydrolysis with $N HCl$. Moreover one of the three labile phosphoric acid molecules may be set free on precipitation of the dinucleotide as a lead salt, giving then an adenosinediphosphate with a ratio of labile to stable P of 1 such as Lohmann observed. An example of this change having taken place may be given. A portion of the calcium salt of the cardiac derivative (0.2 g.) after removal of calcium was precipitated with normal lead acetate, the lead salt decomposed in the usual way and the solution made up to the same volume as before precipitation. The phosphorus distribution before and after precipitation by lead was as follows (mg. P per ml.).

	Before		After	
Orthophosphoric acid	nil	0.064	orthophosphoric	0.064
After 10 mins. acid hydrolysis	0.177	0.177	labile	0.113
Total	0.290	0.290	stable	0.113

In the solution prior to precipitation with lead 61 % of the total P was set free on acid hydrolysis ($N HCl$), corresponding therefore to 3 labile to 2 stable groups as in an equimolecular mixture of the tri- and di-phosphates. If the solution had contained the triphosphate alone, 66 % of the total P would have been in labile form.

Acid hydrolysis of the cardiac nucleotide.

In all such hydrolysis experiments with $N HCl$ special attention requires to be directed to the amounts of inorganic phosphate set free within the first 10–30 mins., because, subsequent to the setting free of the labile P, the rate of hydrolysis of the stable P is very slow and for all such adenyl nucleotides as occur in muscle fairly constant.

The procedure adopted was as follows. One ml. of the solution containing a known quantity of the cardiac (or skeletal) derivative was mixed with 1 ml. of 2*N* HCl or 2*N* acetic acid and kept at 100° in sealed tubes for the periods stated.

After rapid cooling the solutions were neutralised and made up to a volume corresponding to the standard solution concentration (0.4 mg. P per 100 ml.), so that the colorimetric determinations might be carried out under strictly comparable conditions.

Table I. *Hydrolysis in N HCl.*

Skeletal muscle adenosinetriphosphoric acid.			
Time (mins.)	(1) P as orthophosphate mg. per ml.	(2) % of total P	(3) % of labile P (2 in 3)
0	0		
10	0.137	67	100
15	0.137	67	
30	0.140	68	
60	0.140	71	
	Total P	0.204	
Cardiac muscle dinucleotide.			
Time (mins.)	(1) P as orthophosphate mg. per ml.	(2) % of total P	(3) % of labile P (3 in 5)
0	0		
10	0.123	60	100
15	0.131	64.5	
30	0.137	67.4	
60	0.141	69.4	

Table II. *Hydrolysis in N acetic acid.*

Skeletal muscle adenosinetriphosphoric acid.			
Time (mins.)	(1) P as orthophosphate mg. per ml.	(2) % of total P	(3) % of labile P
0	0		
10	0.020	9.8	14.7
15	0.030	14.7	22.0
30	0.046	22.5	33.8
60	0.073	35.2	53.6
120	0.125	61.2	91.8
210	0.136	67.0	100.0
	Total P	0.204	Labile P 0.136
Cardiac dinucleotide.			
Time (mins.)	(1) P as orthophosphate mg. per ml.	(2) % of total P	(3) % of labile P
0	0		
10	0.036	8.5	14.2
15	0.046	11.1	18.2
30	0.103	24.5	40.8
60	0.143	34.0	56.7
120	0.195	46.4	77.3
210	0.252	61.4	100.0
	Total P	0.420	Labile P 0.252

The skeletal nucleotide loses 2/3 of its P after 10 mins. hydrolysis with *N* HCl, while from the cardiac one under the same conditions 3/5 of the total P is set free. On hydrolysis with *N* acetic acid the same changes take place after boiling for

210 mins. After 120 mins. the proportion of the loosely bound P split off from the cardiac derivative is distinctly less than from the skeletal one.

Alkaline hydrolysis of the cardiac dinucleotide.

Lohmann and others have frequently referred to the liberation of ortho-phosphate or pyrophosphate from the breakdown of adenosinetriphosphoric acid by $\text{Ca}(\text{OH})_2$ and $\text{Ba}(\text{OH})_2$. It is often impossible to be certain from statements in the literature whether the precipitates of so-called calcium or barium pyrophosphates were purely inorganic or not.

Precipitates obtained after half an hour's hydrolysis in $\text{Ca}(\text{OH})_2$ always contain a fairly high percentage of N, although the main product, adenylic acid, forms a soluble calcium salt.

Mixtures of one volume of the solutions of the two nucleotides with one volume of saturated $\text{Ca}(\text{OH})_2$ were kept at 100° in sealed tubes for the periods stated, and the orthophosphate at each stage was determined. The mixtures were shaken throughout.

The percentage of labile P in the adenosinetriphosphoric acid is calculated as 66 % of the total, that of the dinucleotide as 60 %.

Table III.

Time (mins.)	Skeletal adenosinetriphosphoric acid			Cardiac dinucleotide		
	1	2	3	1	2	3
	P as ortho-phosphate mg. per ml.	% of total P	% of labile P	P as ortho-phosphate mg. per ml.	% of total P	% of labile P
0	0					
5	0.029	30.2	45.3	0.043	43.0	71.6
10	0.047	48.9	73.4	0.045	45.0	75.0
15	0.047	48.9	73.4	0.047	47.0	78.3
30	0.049	51.0	76.5	0.047	47.0	78.3
90	0.066	68.7	100.0	0.050	50.0	83.3
120	0.066	68.7	100.0	0.064	64.0	100.0
180	—	—	—	0.064	64.0	—
	Total P	0.096		Total P	0.100	

Difficulties arise in the determination of the rates of hydrolysis with $\text{Ca}(\text{OH})_2$ owing mainly to the fact that the salts to be acted upon are not in a state of solution but in one of fine suspension. After the loosely bound phosphoric acid molecules have been set free, the calcium salt of adenylic acid along with varying amounts of adenosine should pass into solution. As it is the amount of ortho-

Table IV.

Time (mins.)	P as orthophosphate mg. per ml.	%
0	0	
5	0.077	48.1
10	0.102	63.6
15	0.130	81.2
30	0.130	81.2
90	0.160	100.0
120	0.160	
Total P	0.160	

phosphate which is being estimated at the different stages, the rate of hydrolysis of the inorganic pyrophosphate by $\text{Ca}(\text{OH})_2$ requires to be known as the loosely bound phosphorus in the nucleotides may be set free in this form. Table IV gives the results of the hydrolysis of sodium pyrophosphate with $\text{Ca}(\text{OH})_2$.

When the results of the hydrolysis of the three compounds are examined, it is evident that one factor which may determine the rate of orthophosphate formation from the organic substances is the rate of change of pyrophosphate into orthophosphate. That this is not the only factor in the case of the dinucleotide is evident from the greater initial rate at which orthophosphate is set free from it compared with inorganic pyrophosphate. The labile portion of adenosinetriphosphoric acid behaves somewhat similarly to the inorganic salt on alkaline hydrolysis, just as it does in the case of hydrolysis with *N* HCl. At present no definite evidence is obtainable regarding the position of the labile phosphoric acid molecules in these nucleotides, but it appears probable that there is an anhydrodiphosphoryl linkage in the triphosphoric acid which may combine in the case of dinucleotides with a secondary alcohol group of the ribose in either an adenosine-monophosphate or diphosphate.

II. THE BIOLOGICAL AND CO-FERMENT PROPERTIES OF THE CARDIAC DINUCLEOTIDE.

THE definite biological actions of the adenosinetriphosphoric acid of skeletal muscle origin and the function of that nucleotide in lactic acid production suggest that an investigation into the pharmacology and co-ferment properties of the cardiac dinucleotide would be of special interest for several reasons. In the first place the substance is a natural component of the heart-muscle, and as such its actions on the heart and vessels are of importance, and in the second place, as chemically it presents certain characteristic differences from the derivative of skeletal muscle, any difference in action between the two might be explicable from the standpoint of chemical constitution. Finally, as proprietary preparations of tissue nucleotides and nucleosides are in routine use in the treatment of cardio-vascular disease, any further investigation into the pharmacology of such substances is of value.

Drury and Szent-Györgyi [1929] have studied the biological actions of a cardiac derivative which, as they deduced from careful analyses, was undoubtedly adenylic acid. They refer only briefly to the action of Fiske's adenosinetriphosphate (probably from skeletal muscle).

In all the following experiments the calcium salt of the cardiac dinucleotide was used.

The results are exactly comparable with those of Gillespie [1933] for the skeletal muscle nucleotide, in most cases both salts being tried on the same preparation.

Biological actions.

1. *The heart.* (a) Doses of 1 in 50,000 of the cardiac nucleotide perfused through the isolated frog's heart cause no alteration in the normal beat. Doses of 1 in 25,000 produce an A.V. block which passes off on perfusion with fresh Ringer's solution.

(b) In the isolated perfused rabbit's heart, doses of 0.1 to 0.2 mg. of the heart dinucleotide added to the perfusion fluid cause first a diminution in rate and amplitude of the beat followed in a few seconds by augmentation of the beat and an increased coronary outflow.

2. *Blood pressure.* Doses as small as 0.01 mg. lower the carotid arterial pressure of the rabbit. The fall in pressure is not so sudden as that produced by vagal stimulation. The fall is preceded by a short rise. The whole effect is quite transitory, and complete recovery takes place in a few seconds. This was also seen after atropine.

3. *Perfusion of vessels.* Frogs' vessels were perfused in the ordinary way. Doses of 0.2 mg. injected at the point of entrance of the perfusion fluid doubled the drop rate in about $1\frac{1}{2}$ mins.

4. *Uterus.* Concentrations of 1 in 250,000 of the cardiac dinucleotide increase the tone of the virgin guinea-pig's uterus perfused by the Sawasaki technique and finally give rise to spontaneous contractions. This is the same effect as is described by Deuticke [1932] in connection with Embden's heart nucleotide.

5. *Intestine.* Concentrations of 1 in 50,000 and less lower the tone of the perfused small intestine of the rabbit. This is a different effect from that produced by the voluntary muscle nucleotide, which causes a marked increase in tone immediately after the fall [Gillespie, 1933]. Using the intestine as a test object it was shown that dialysates of saline extracts of heart muscle owe their biological action to the dinucleotide component.

The action of the cardiac dinucleotide on the amphibian and mammalian heart and vessels and on the perfused uterus closely resembles, both qualitatively and quantitatively, the action of the voluntary muscle nucleotide as described by Gillespie. Voluntary muscle nucleotide produces a fall in intestinal tone followed by a marked rise. Only the fall has been observed with the cardiac derivative.

Ferment actions of the cardiac nucleotide.

The glycolytic ferment of voluntary muscle consists of two fractions—the colloidal ferment and a co-ferment which Meyerhof and Lohmann [1928] have identified with adenylypyrophosphate. Little is known of the glycolytic ferment system in the heart, and as the dinucleotide just described has been tested as co-ferment on cardiac extracts acting on artificial substrates of starch or glucose with orthophosphate, the results of this investigation help to complete the comparison of the two types of nucleotide. Lambs' hearts fresh from the abattoir were used for these experiments.

The first experiments show that an active ferment system can be extracted from the mammalian heart muscle with ice-cold saline. This activity is shown by the appearance of lactic acid in the incubation mixture containing starch and inorganic phosphate and by the disappearance of some of the orthophosphate due to the synthesis of esters. For example:

2nd and 3rd saline extracts incubated 2 hrs. at 25° with starch substrate.

m.mols ester produced	...	...	...	5.6
m.mols lactic acid produced	...	...	...	5.0

When the extract has been kept for 24 hrs. at 4° it no longer produces these effects. The addition of a small amount of the cardiac dinucleotide, or the

adenosinetriphosphoric acid of skeletal muscle, restores the activity of the extract. The following experiment will illustrate the type of action.

2nd and 3rd saline extracts, kept 24 hrs. at 4°, incubated 3½ hrs. at 25° with starch substrate.

1. Extract + substrate + no addition.

m.mols ester produced	...	...	...	0.39
m.mols lactic acid produced	...	...	...	nil

2. Extract + substrate + 0.1 mg. skeletal adenosinetriphosphoric acid per ml.

m.mols ester produced	...	...	...	0.70
m.mols lactic acid produced	...	...	...	0.79

3. Extract + substrate + 0.1 mg. cardiac dinucleotide per ml.

m.mols ester produced	...	...	...	2.10
m.mols lactic acid produced	...	...	...	2.00

The cardiac dinucleotide reactivates the extract to a greater degree than does the nucleotide of voluntary muscle. It was found that this occurred also in the case of the reactivation of voluntary muscle extracts. Haarman [1932], in a paper published while these experiments were being carried out, points out that the preferential substrate for heart glycolysis is glucose. Again using the extract described above, but with glucose in place of starch as substrate, the results obtained were as follows:

1. Extract + substrate (glucose) + no addition.

m.mols ester produced	...	...	...	0.6
m.mols lactic acid produced	...	...	...	0.1

2. Extract + substrate (glucose) + 0.1 mg. skeletal muscle adenosinetriphosphoric acid per ml.

m.mols ester produced	...	...	...	0.7
m.mols lactic acid produced	...	...	...	1.0

3. Extract + substrate (glucose) + 0.1 mg. cardiac dinucleotide per ml.

m.mols ester produced	...	...	...	0.0
m.mols lactic acid produced	...	...	...	2.0

In these and other examples it was seen that the extract did not show a preference for glucose, though the observation was confirmed that the heart could use this substrate. It is evident, however, that while esterification is a feature of lactic acid production from starch, this is not the case when the substrate is glucose. Traces of ester may be found with glucose as substrate due to the glycogen content of the original extract. When minced heart muscle is added to an artificial substrate, it is found that, while starch can be used, there is a definite preference for glucose. As in the case of the saline extracts there is no tendency for esters to accumulate when the glucose substrate is used:

3 g. specimens of minced muscle were allowed to act on the phosphate, glucose and starch substrates in a total volume of 20 ml. for 2½ hrs. at 25°.

1. Glucose substrate.

Orthophosphate used up	...	...	0.52 mg. P in 20 ml.
Lactic acid produced	...	...	1.92 mg. in 20 ml.

2. Starch substrate.

Orthophosphate used up	...	...	2.14 mg. P in 20 ml.
Lactic acid produced	...	...	0.60 mg. in 20 ml.

It also has been found that a more powerful ferment system can be extracted from muscle which has been autolysed for 2 hours than from fresh muscle. The extract of autolysed muscle can be made still more active by addition of co-ferment. It is evident that during autolysis the colloidal component of the ferment system undergoes some change which facilitates its extraction.

The ferment system of cardiac muscle is, on the whole, not nearly so active as the corresponding system in voluntary muscle.

That glucose should be available as an immediate source of energy for the heart, whether or not glycogen is present, is of great interest. The glycolytic ferment system of voluntary muscle is incapable of using glucose directly. This means that, apart from the smooth muscle tissues with their slow rate of metabolism, the heart has an option on sugars in the circulation, a mechanism which must be of considerable importance in maintaining the heart beat in cachexia and privation when glycogen is low. It may also explain in part the therapeutic value of parenteral glucose saline in the treatment of shock and allied conditions.

SUMMARY.

1. The nucleotide of cardiac muscle differs chemically from the adenosinetriphosphoric acid of voluntary muscle, being more closely allied to a dinucleotide composed of one molecule of adenosinediphosphoric acid and one molecule of adenosinetriphosphoric acid.

2. This is shown by (a) its chemical composition and (b) its behaviour on hydrolysis at 100° with *N* HCl. Approximately 60 % of its total P is in the labile form in place of 66–67 % in adenosinetriphosphoric acid.

3. The pharmacological actions are very similar to those of the voluntary muscle derivative, the only distinctive difference being observed in the reaction of the intestinal musculature, the dinucleotide lowering the tone without a subsequent rise, while adenosinetriphosphoric acid has the double action.

4. The heart dinucleotide has a more powerful co-ferment action as regards the reactivation of inactive ferment extracts both of voluntary and cardiac muscle.

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