L'activité ATPasique est accrue entre le 2e et le 4e jour après l'irradiation, à une exception près (Tableau II). Au-delà du 4e jour, les différences ne sont pas homogènes. L'augmentation de l'activité ATPasique constatée les premiers jours après l'irradiation est en accord avec les faits observés par ASHWELL et HICKMANN[4] et VAN BEKKUM[5] au niveau de la rate chez la souris blanche et par DUBOIS et PETERSEN[6] au niveau de la rate et du thymus chez le rat et la souris mâle. Cette majoration de l'activité ATPasique pourrait être rapprochée d'un accroissement similaire mis en évidence dans le cristallin d'animaux diabétiques[7]. Nous l'avons interprété dans ce cas comme une adaptation enzymatique tendant à contrebalancer certains blocages de la dégradation des glucides. Il pourrait en être de même dans les suites après irradiation. Notons encore que le comportement des deux enzymes étudiés *in vivo* s'avère nettement différent de celui *in vitro*. Dans ce dernier cas, on a signalé en effet que l'hexokinase résiste mieux aux rayons X que l'ATPase.

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Summary

The irradiation of the lens by a simple dose of 1400 r, gives a very important and lasting reduction of its hexokinase activity. Its ATPase activity shows an increase during the first four days and a return to normal again. The variations observed here (that is *in vivo*) are different from the ones noted after irradiation of these enzymes *in vitro*.

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ATPase Activity of Guinea Pig Heart Muscle

The occurrence of several different types of enzymes splitting ATP in animal tissue has been recently described. Biochemical differences among those enzymes concern principally pH optimum, stimulating cations and reaction products. Quite recently, HEPPEL and HILMOE[1] have succeeded in separating three different ATPases from bull seminal plasma. One of these enzymes hydrolyzes ATP to AMP and PP. It is relatively heat-stable, has pH optimum between 8·4 and 8·8 and requires neither Ca^{++} nor Mg^{++} for full activity. The second ATP-splitting enzyme produces *ortho* P and ADP from ATP and has an acid pH optimum. It is completely destroyed by heating at 60°C for 20 min, requires Mg^{++} and is inhibited by Ca^{++}. The third enzyme is a relatively heat-stable alkaline phosphatase which releases *ortho* P from ATP and is more stimulated by Ca^{++} than by Mg^{++}.

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The importance of ATP and ATPase in muscular contraction has been particularly emphasized in recent years (SZENT-GYÖRGYI[2], MOMMAERTS[3], NEEDHAM[4]) Some features of muscular ATPase have been then extensively studied. All the above-mentioned authors agree that muscular ATPase shows two pH optima for enzymic activity: the first one is near pH 6·5; the second is much stronger and develops in the presence of Ca^{++} at pH 9·2. Ca^{++} acts as an activator at both pH values; the action of Mg^{++} is, by contrast, still a matter for discussion. BANGA and SZENT-GYÖRGYI[5] have discovered that pure myosin and actomyosin with lower actin contents are always inhibited by Mg^{++}, whereas actomyosins with higher actin contents can be activated.

The purpose of the present investigation was to study the characteristics of ATPase from guinea pig heart muscle. In order to establish it there are differences in enzymic activity in regions of the heart provided with different functions, ATPase activity of both ventricles and atria were studied separately.

Methods.—Male guinea pigs weighing 250–300 g and fed on a standard diet were used. The animals were killed by bleeding and the heart was immediately removed and transferred into the cold room at 2°C. Both ventricles and atria were separated and weighed. 1% homogenates were then prepared by grinding the minced tissues in a Potter-Elvehjem homogenizer with a leucite pestle. 0·067 *M* borate buffer was used as a suspension medium.

ATPase activity was determined according to the method of DUBOIS and POTTER[6] with some modifications described elsewhere[7]. Two pH optima indicated by MOMMAERTS (6·5 and 9·2) were particularly investigated. Incubation temperature was 37°. ATP sodium salt was prepared in solution from dibarium salt supplied by SCHWARZ. Its final concentration was 0·01 *M*. The values reported in the Tables are given as µg P/mg N. P. determinations were made according to the method of FISKE and SUBBAROW[8] and N determinations by the usual microkjeldhal technique. The values obtained were analyzed statistically, standard deviation and the "t" test of STUDENT-FISHER being calculated for each average.

Results.—In a first group of experiments the normal values of ATPase of atria and ventricle homogenates of guinea pig heart were established. ATPase activity was studied without the addition of activating cations at two pH optima 6·8 and 9·2. As shown in Table I, ATPase activity is much stronger at pH 9·2 than at pH 6·8, as the enzyme from skeletal muscle does. No remarkable differences in activity of different parts of the heart were noticed at pH 9·2. At pH 6·8 the activity of the atria is, however, stronger than that of the ventricles.

In a second group of experiments the influence of Ca^{++} and Mg^{++} was studied. The results are represented in Fig. 1 and 2. It is clear that the action of both ions depends upon their concentration and that the activity maximum corresponds always to a low concentration, whereas at

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Table I

Influence of $CaCl_2$ and $MgCl_2$ on ATPase activity at pH 6·8 and 9·2. The values are given as μg P/mg N delivered in 15 min at 37° C, ± standard deviation. 10 experiments with $CaCl_2$, 8 with $MgCl_2$.

Activator added	Activator concentration	pH 6·8				pH 9·2			
		L. ventr.	L. atria	R. ventr.	R. atria	L. ventr.	L. atria	R. ventr.	R. atria
— — —	— — —	48 ± 5	79 ± 4	58 ± 3	85 ± 7	103 ± 6	122 ± 8	140 ± 11	127 ± 9
$CaCL_2$	$9{\cdot}6 \times 10^{-4}$	59 ± 4	87 ± 4	73 ± 5	91 ± 5	150 ± 6	131 ± 6	180 ± 5	143 ± 7
$CaCl_2$	$1{\cdot}6 \times 10^{-3}$	50 ± 2	99 ± 4	58 ± 4	110 ± 6	139 ± 4	125 ± 5	178 ± 6	133 ± 6
$CaCl_2$	$3{\cdot}3 \times 10^{-3}$	50 ± 4	75 ± 5	64 ± 4	85 ± 3	141 ± 5	137 ± 5	167 ± 5	148 ± 5
$CaCl_2$	5×10^{-3}	50 ± 2	–	55 ± 3	–	105 ± 4	160 ± 6	132 ± 4	171 ± 6
$CaCl_2$	$6{\cdot}6 \times 10^{-3}$	50 ± 3	78 ± 3	57 ± 2	87 ± 4	111 ± 5	135 ± 5	141 ± 5	144 ± 3
$CaCl_2$	$8{\cdot}3 \times 10^{-3}$	50 ± 4	80 ± 3	58 ± 3	89 ± 4	110 ± 5	116 ± 4	144 ± 3	124 ± 4
$MgCl_2$	$9{\cdot}6 \times 10^{-4}$	79 ± 5	95 ± 6	85 ± 5	130 ± 5	152 ± 4	167 ± 8	190 ± 8	179 ± 7
$MgCl_2$	$1{\cdot}6 \times 10^{-3}$	63 ± 3	105 ± 5	70 ± 5	136 ± 6	140 ± 6	145 ± 6	188 ± 5	175 ± 7
$MgCl_2$	$3{\cdot}3 \times 10^{-3}$	50 ± 3	80 ± 4	76 ± 3	122 ± 5	154 ± 6	125 ± 6	170 ± 6	156 ± 6
$MgCl_2$	5×10^{-3}	46 ± 4	77 ± 5	60 ± 4	129 ± 7	169 ± 8	140 ± 8	165 ± 7	150 ± 9
$MgCl_2$	$6{\cdot}6 \times 10^{-3}$	35 ± 3	68 ± 4	57 ± 3	120 ± 6	150 ± 8	135 ± 7	165 ± 7	150 ± 8
$MgCl_2$	$8{\cdot}3 \times 10^{-3}$	44 ± 3	80 ± 6	62 ± 3	110 ± 5	110 ± 7	120 ± 4	168 ± 9	152 ± 7

higher values the activation disappears. Some difference of behaviour between atria and ventricles was observed also with respect to Ca^{++} and Mg^{++} stimulation at different pH values.

In the case of Ca^{++} and pH 6·8 (Table I and Fig. 1) the curves of two atria and ventricles are very similar: they start from low values, rise slightly with increasing Ca^{++} concentration until they reach a maximum at 1×10^{-3} *M*, and then descend abruptly towards rather constant values which are somewhat lower than those observed in the absence of added Ca^{++}. The maximum of activity of ventricular ATPase is only slightly displaced towards lower concentrations of Ca^{++}, with respect to that observed in the case of atrial ATPase. Furthermore, the right ventricle shows a second maximum at higher concentrations. At pH 9·2 (Table I and Fig. 1) also the Ca^{++}

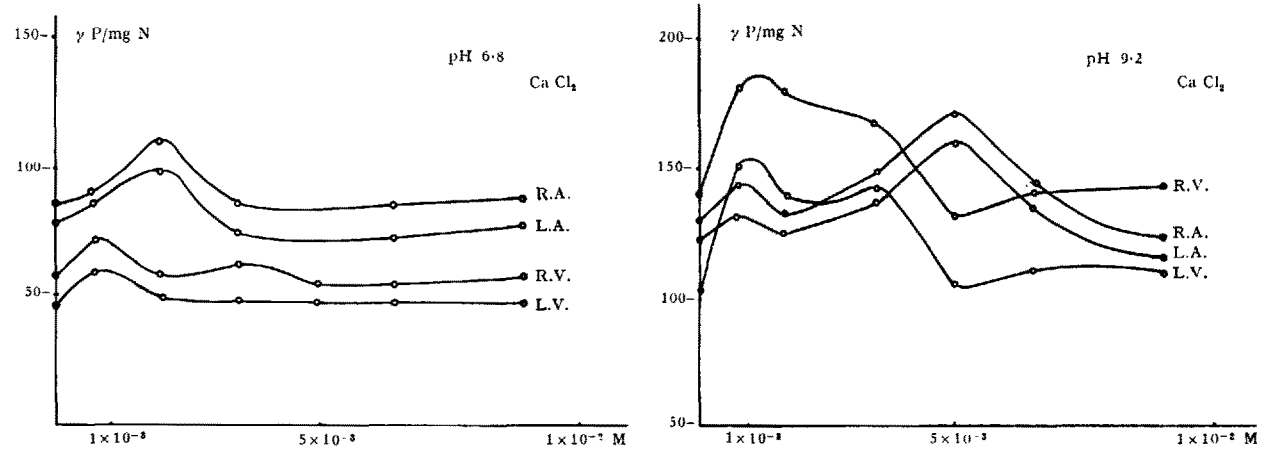

Fig. 1.–Influence of $CaCl_2$ at pH 6·8 and 9·2

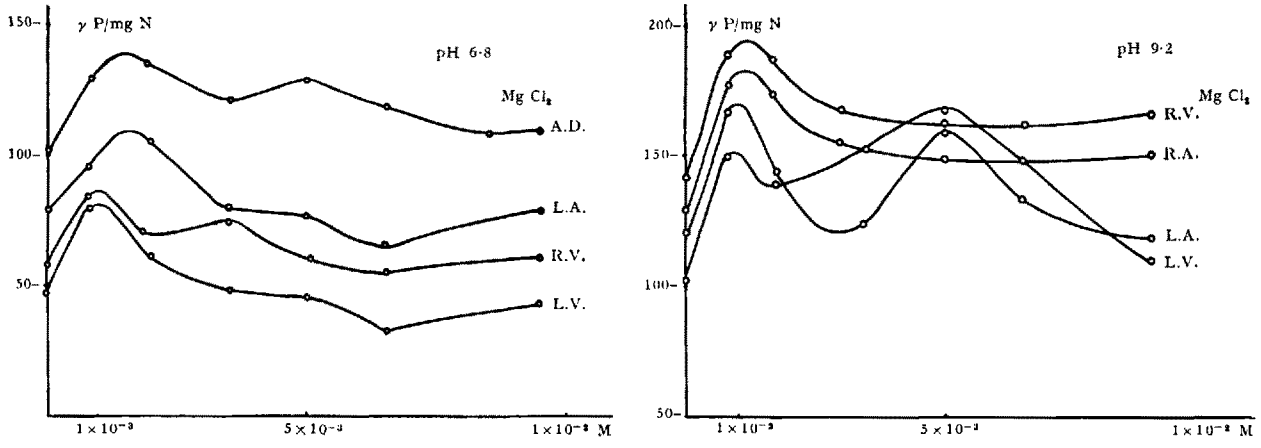

Fig. 2.–Influence of $MgCl_2$ at pH 6·8 and 9·2

Table II
Influence of heating at 60° C (pH 6·8 and 9·2). 5 experiments.

min	pH 6·8				pH 9·2			
	L. ventr.	L. atria	R. ventr.	R. atria	L. ventr.	L. atria	R. ventr.	R. atria
5	40 ± 4	75 ± 4	50 ± 4	75 ± 3	77 ± 5	92 ± 5	112 ± 6	102 ± 6
10	26 ± 2	50 ± 3	37 ± 3	50 ± 3	51 ± 4	62 ± 3	78 ± 2	62 ± 2
15	18 ± 2	40 ± 2	24 ± 2	40 ± 2	26 ± 2	48 ± 2	38 ± 3	38 ± 3
20	11 ± 3	22 ± 2	12 ± 1	19 ± 1	2 ± 1	15 ± 3	5 ± 2	5 ± 2

has a different action on ATPase activity of atria and ventricles. In all four curves maximum values occur at low concentrations, but the forms of the curves show striking differences between atria and ventricles. In fact, ventricles show only one maximum, while the atria have two maxima: a little one occurring at low concentrations, and a second which is high and wide and occurs at high concentrations. Mg^{++} exerts very similar influences on both left atrium and left ventricle as well as on right atrium and right ventricle.

In the presence of Mg^{++} at pH 6·8 right atrium and right ventricle show two well-defined maxima, while left ventricle and left atrium have only one. (Table II and Fig. 2). At pH 9·2, on the other hand, left ventricle and left atrium have two maxima, whereas right atrium and ventricle have only one maximum at low concentrations (Table I and Fig. 2).

In the last group of experiments the extent of destruction of enzyme after heating at 60° was studied. The homogenates were incubated at 60°; small aliquots were taken up at 5 min intervals and ATPase activity was determined. The results are shown in Table II. It is clear from this Table that heart ATPase is heat-labile and is completely destroyed in 20 min at 60°. No difference of behaviour between atria and ventricles was observed.

Conclusions.—It seems probable from these investigations that ATPase activity of guinea pig heart results from the sum of at least two different enzyme activities, each provided with different cation requirements. The first is active at pH 6·8, is stimulated more by Ca^{++} than by Mg^{++} and is especially abundant in the atria; the second is stimulated by Ca^{++} as well as by Mg^{++} in about the same amounts and seems to be most important in ventricles. Ca^{++} stimulation reveals striking differences between the activites of atria and ventricles at both pH; Mg^{++} stimulation seems to exert different actions in the right and in the left heart. However, inactivation by heating proceeds in the same manner in all parts of the guinea pig heart. It seems not improbable that the differences of ATPase activity occurring in atria and in ventricles are due to their functional differences.

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Riassunto

L'Autore ha studiato l'influenza della concentrazione degli ioni Ca^{++} e Mg^{++} sull'ATPasi degli atri e dei ventricoli di cuore di cavia. Tale attività è maggiore a pH 9,2 che a pH 6,8. Mentre il Ca^{++} influisce differenziando decisamente le attività degli atri da quelle dei ventricoli, il Mg^{++} accentua piuttosto una differenza tra la parte destra e la sinistra. Il calore distrugge quasi completamente l'attività enzimatica dopo una permanenza a 60° per 20 min.

Quantitative Determination of Acetylcholinesterase Activity in Individual Megakaryocytes at Various Stages of Maturation

It has been shown that the enzyme acetylcholinesterase (AChE), present in man in the erythrocyte-erythropoietic cells, in rabbit, rat and cat is concentrated in the platelet-megakaryocytes[1]. In a previous report the AChE of the megakaryocytic cell system was investigated with a histochemical technique[2]. It was thereby shown that the megakaryoblast, which has a diameter of about 24 μ and is the earliest-known precursor cell of megakaryocytes, contains considerable amounts of AChE. The strongest histochemical reaction, however, was exhibited at the promegakaryocyte-megakaryocyte stage when the cells attain a diameter of about 42 to 56 μ. In a suspension of bone-marrow cells these maturation stages of megakaryocytes can readily be differentiated by light microscopy. This cell system, therefore, constitutes an ideal medium for studying the problem of the synthesis of a specific enzyme during the maturation of the somatic cells.

Very recently a micro diver technique has been evolved[3], with which it is possible to make quantitative determination of the AChE activity in single cells. Mature megakaryocytes from different mammals were studied in this way[4] as were nerve cells[5]. The present paper reports quantitative determination of AChE activity in individual megakaryocytes at various stages of maturation and isolated from rat bone marrow. The enzyme activity is expressed as the amount of CO_2 evolved in 1 h from the bicarbonate buffer by the acetic acid formed in the enzymatic hydrolysis of acetylthiocholine (AThCh).

The AChE activity of individual megakaryocytes at various stages of maturation is shown in Figure 1. It is seen that even megakaryoblasts, which are about 24 μ in diameter, show measurable amounts of AChE. At the megakaryoblast-promegakaryocyte stage (24–42 μ) the cells show widely varying AChE content. Most cells are very active in splitting AThCh, but some show remarkably low AChE activity. At these developmental stages the megakaryocytes also display great variations in intracellular structure. All intermediate forms ranging from cells with a large, round nucleus surrounded by small amounts of coarse cytoplasmic matter to cells with a polymorphic nucleus and finely granulated cytoplasm were encountered. Attempts to correlate the various levels of AChE activity with particular cell structures, however, have so far been unsuccessful.

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