*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 $\mu$ 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 $\mu$. 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 $\mu$ in diameter, show measurable amounts of AChE. At the megakaryoblast-promegakaryocyte stage (24–42 $\mu$) 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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Megakaryocytes which had reached a diameter of about 45 $\mu$ all show a polymorphic nucleus surrounded by a large disc of homogeneous, finely granulated cytoplasm. In studying individual megakaryocytes it was noted that at the megakaryoblast-promegakaryocyte stage spherical cells became flattened in the final phases of maturation. This process suggests a decrease of the cell mass per cross-section area and also that the increased size of megakaryocytes in the final stages of maturation may not entail an increase of the total cell mass. Recent interferometric mass determinations[6] have in fact shown that the amounts of dry substance per $\mu^2$ area are much lower in the mature (largest) megakaryocytes ($0{\cdot}57 \times 10^{-12}$ g/$\mu^2$) than in the smaller cells ($0{\cdot}82 \times 10^{-12}$ g/$\mu^2$). Figure 1, however, shows that in the final maturation stages rapid enhancement of AChE activity takes place. It is thus possible that this represents a process similar to that discussed by THORELL[7] in regard to the synthesis of haemoglobin, which was considered to begin mainly after the formation of basic cellular protein-substances is accomplished.

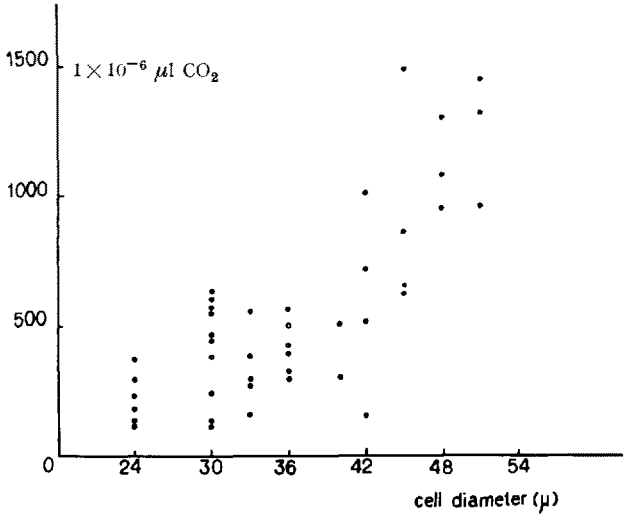


Fig. 1.–Acetylcholinesterase activity of individual megakaryocytes at various stages of development. The enzyme activity is expressed as $1 \times 10^{-6}$ µl $CO_2$ evolved from the substrate bicarbonate solution in one h.

The variability of enzyme content in megakaryocytes of apparently similar degree of maturation (shown in Fig. 1) can also readily be demonstrated by histochemical means. In Figure 2 a modified[1] thiocholine method for AChE determination[8] has been applied to a group of megakaryocytes at the 30–36 $\mu$ stage. The megakaryocyte at the top of the figure shows high AChE activity, while a similar cell in the right lower corner displays only slight histochemical reaction. After being photographed, the megakaryocytes in Figure 2 were washed in 0·9% NaCl and placed in divers for quantitative determination of AChE. The cell with high histochemical activity was found to liberate from the substrate bicarbonate solution about $550 \times 10^{-6}$ µl $CO_2$ per h and the cell with little histochemical activity only about $120 \times 10^{-6}$ µl. Whether the low AChE activity of the second cell was due to slower enzymatic synthesis or to temporary inactivation of the enzyme–active centra being combined with other molecules (templates) in order to reduplicate themselves–is not known, and cannot be elucidated from the data herein presented.

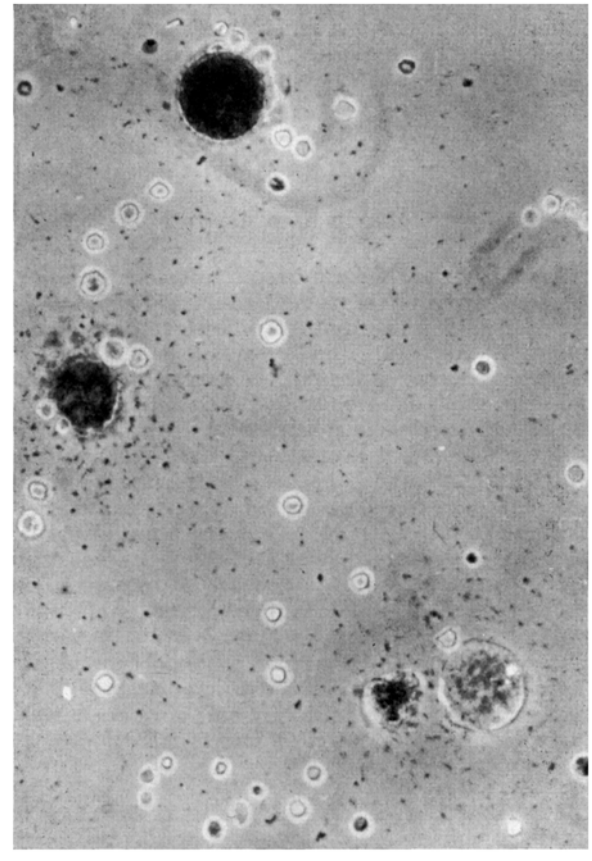

Fig. 2.–Histochemical demonstration of AChE activity in a group of megakaryocytes of rat bone marrow 30 to 36 $\mu$ in diameter. The megakaryocyte in the upper section of the photograph shows a highly positive histochemical reaction, whereas a similar cell in the right lower corner appears to have little activity (phase-contrast × 350).

The ribonucleic acids (RNA) have repeatedly been suggested as the template or pattern for specific protein synthesis[9]. Ultraviolet photometry of megakaryocytes at various stages of development has shown that parallel with maturation a continous decrease of cytoplasmic nucleotide substances takes place[6]. The micromethod developed by EDSTRÖM[10] for RNA determination in individual nerve cells has now been applied to megakaryocytes. The preliminary data indicate that the RNA concentration in megakaryocytes decreases greatly during maturation stages in which the rapid increase of AChE activity takes place. The possible relationship between the synthesis of AChE and the metabolic processes of RNA is under investigation.

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*Zusammenfassung*

Die Aktivität der Azetylcholinesterase von einzelnen Megakaryozyten verschiedener Reifegrade wurde mit Hilfe des Cartesischen Tauchers bestimmt. Die Megakaryozyten zeigen auf dem Megakaryoblasten- und Promegakaryozytenstadium grosse Unterschiede der Azetylcholinesteraseaktivität. In der letzten Phase der Reifung, wenn die Zellen einen Durchmesser von 45 $\mu$ erreichen, wird das Ferment am stärksten wirksam.

## Post-heparin Esterase in Man

It is known that heparin has an influence on the enzymatic activity of serum and plasma. The nature of these changes has already been investigated[1]. In the investigation described, the effect of heparin on the esterolytic activity of serum in man was observed. The esterase level was determined by the titrimetric method[2], using ethyl butyrate as substrate. Human blood serum was investigated before and 10–15 min after the injection of 5000 units of heparin intravenously. An increase of from 10 to 100% was found in 30 examinations. This finding was observed in both serum and plasma; heparin, however, acted only *in vivo*. When substrates other than ethyl butyrate were used, an increase was also observed with ethyl isobutyrate; with other esters, e.g. ethyl acetate, isopropyl acetate, butyl acetate, amyl acetate, triacetin and tributyrin, the increase was small or absent. The level of esterase rose as early as 2 min after injection, it then gradually fell; however a higher value was still evident after 60 min (Table I).

*Table I*

| Time after injection minutes | Esterase ml 0·05 *n* NaOH |
|---|---|
| 0 | 2·72 |
| 2 | 4·00 |
| 20 | 3·68 |
| 40 | 3·52 |
| 60 | 3·32 |

*Table II*

| Concentration of NaF | Pre-heparin esterase ml 0·05 *n* NaOH | Post-heparin esterase ml 0·05 *n* NaOH |
|---|---|---|
| – | 2·68 | 3·56 |
| 0·001 m | 1·62 | 2·98 |
| 0·01 m | 0·72 | 2·36 |
| 0·1 m | 0·18 | 1·60 |
| 1 m | 0·08 | 0·32 |

Post-heparin esterase acts at pH 7–11, a higher pH is more favourable to it. It is resistant to various inhibitors, e.g. NaF (Table II), physostigmine ($10^{-3}$ m), diethyl-*p*-nitrophenylphosphate ($10^{-5}$ m). Similarly, it shows a somewhat greater resistance to heat than the esterase of normal serum. Sodium taurocholate decreases its effectiveness. Similarly, protamine inhibits it *in vitro*. The effect of protamine, however, can be eliminated by the addition of heparin in excess.

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*Zusammenfassung*

Es werden einige Eigenschaften der menschlichen Serumesterase nach Heparinapplikation beschrieben.

## Tubular Factors in the Renal Response to Arterial Hypotension

The extreme oliguria in posthaemorrhagic hypotension is well known[1]. In a previous communication[2] we were able to demonstrate that the posthaemorrhagic diminution of diuresis is less expressed in the transplanted, i.e. completely denervated kidney, than it is in its innervated partner. So the role of nervous impulses in the mechanism of posthaemorrhagic oliguria seems to be proved. There is no doubt that *one* of the factors responsible for oliguria is the reduced filtration rate (GFR), but an accurate analysis of our data[3] suggested that an increase in tubular reabsorption is also involved in the process.

In a series of experiments performed on dogs under chloralose, innervated and transplanted kidneys were compared for renal bloodflow (RBF), GFR, renal resistance (R = B.P/RBF), and excretion of sodium and water. Part of the experiments were done in osmotic or saline diuresis, part of them without any diuretics at all. RBF was determined directly by cannulating the renal vein. GFR is the product of RPF (determined directly) and the extraction ratio of inulin. Measurements were made both in the basal state, i.e. with arterial pressure normal, and in hypotension induced by constriction of the aorta just above the origin of the renal arteries.

The results are tabulated (arithmetic means with s. d.). In the basal state there was no significant difference between the behaviour of the innervated and transplanted kidneys except for RBF, which was lower; consequently, resistance was higher in the transplanted kidney. After induction of a hypotension of about 70 mm Hg, RBF decreased to about 80% in both series, leading to a decrease of renal resistance. (The hypotensive values are expressed as percentages of the corresponding basal rates.) The response of the renal vessels to hypotension of the lower half of the body was

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