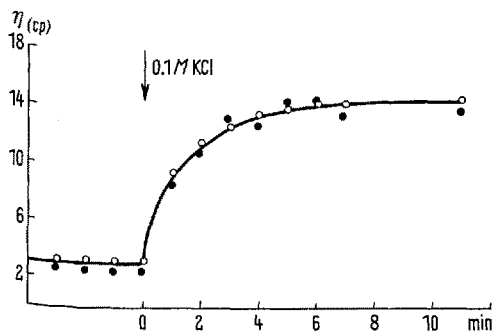
Apparente Sedimentationskoeffizienten wurden in der analytischen Ultrazentrifuge Typ Spinco E mit Phasenplatte und Schlierenoptik ermittelt. Für Einzelheiten sei auf eine frühere Arbeit verwiesen[6]. Die Aminosäureanalyse wurde mit einem Beckman Aminoacid-Analyser durchgeführt. Das Myosinbindungsvermögen von f-Actin errechneten wir nach der Ultrazentrifugenmethode von JOHNSON und ROWE[7].

Resultate. Die Eigenschaften von g-Actin aus Kalbsherzen sind in den beiden Tabellen zusammengefasst. Die Abbildung zeigt die Polymerisation einer g-Actin-Lösung mit und ohne Zusatz von Ouabain. In Anwesenheit von Glykosiden polymerisiertes Actin unterschied sich weder in der Viskosität noch in der Thixotropie von normal polymerisiertem Actin.

Diskussion. Die Parameter von Kalbsherzactin decken sich innerhalb der zu erwartenden Fehlergrenzen mit Werten aus der Literatur für Actin aus Hundeherzen[5] und Kaninchenskelettmuskulatur[8]. Wegen den bekannten technischen Schwierigkeiten beim Arbeiten in verdünnten Salzlösungen haben wir keine physiko-chemische Molekulargewichtsbestimmung durchgeführt. Zusatz von EDTA liess in den Ultrazentrifugenversuchen keinen zweiten Gipfel auftreten. Die Aminosäureanalyse steht in befriedigender Übereinstimmung mit den Werten von CARSTEN und KATZ[9]. Im Gegensatz zu KATZ und HALL[5] finden wir einen niedrigeren Staudingerindex, wofür zum Teil die verschiedene Messtemperatur verantwortlich sein dürfte. Zur Erklärung der Tatsache, dass wir die Polymerisation von Actin nicht durch Glykoside beeinflussen konnten, bieten sich zwei Möglichkeiten an. Entweder ist der Glykosideffekt auf die Polymerisation durch eine Verunreinigung bedingt, oder aber es muss Actin in ganz spezifischer Weise verändert sein, um das Phänomen zu zeigen. Mit der Klärung dieser Frage sind wir beschäftigt[10,11].



Polymerisationsgeschwindigkeit und Viskosität von g-Actin aus Kalbsherzmuskel, 1,4 mg Eiweiss pro ml. ●–●–●– = ohne Glykosidzusatz; o–o–o– = mit Zusatz von 10^{-5} *M* Ouabain.

Summary. Sedimentation coefficient, intrinsic viscosity, amino acid composition and myosin binding capacity of actin from calf's heart were determined. The results are in fair agreement with values published for actin from other sources. No influence of strophantin-g or strophantin-k on the velocity of polymerization and the viscosity and thixotropy of f-actin could be detected.

E. JENNY

Pharmakologisches Institut Universität Zürich (Schweiz), 27. September 1966.

[6] E. JENNY, Helv. physiol. Acta *23*, 357 (1965).
[7] P. JOHNSON und A. J. ROWE, in *Biochemistry of Muscle Contraction* (Ed. J. GERGELY; Little Brown and Company, Boston 1964), p. 279.
[8] M. S. LEWIS und K. A. PIEZ, Abstr. 145th Meeting, New York, Am. chem. Soc., 1963, p. 57 C.
[9] M. E. CARSTEN und A. M. KATZ, Biochem. biophys. Acta *90*, 534 (1964).
[10] Diese Arbeit wurde vom Schweizerischen Nationalfonds zur Förderung der wissenschaftlichen Forschung (Projekt 3046) in dankenswerter Weise unterstützt.
[11] Herrn PD Dr. R. HUMBEL sei für die Durchführung der Aminosäureanalyse herzlich gedankt.

Differential Effect of Oxidized Glutathione or Acetylphenylhydrazine on Individual Electrophoretic Components of Red Cell Acid Phosphatases

The acid phosphatases of the human red blood cells may be resolved in individual components by starch gel electrophoresis, and 6 of the resulting patterns so far described have been interpreted as the phenotypes expected if 3 co-dominant genes (P^a, P^b, and P^c) allelomorphic to an autosomal locus were responsible for their synthesis[1].

In a previous article, we reported that the incubation of an hemolysate with GSSG[2] modifies the electrophoretic pattern of the red cell acid phosphatases with the appearance of faster anodic fractions, followed by a gradual fading of the pattern and a marked decrease in enzyme activity[3]. Similar changes may be induced when the whole red blood cell is incubated with APH[4].

The present report describes a series of experiments suggesting the possibility that the various acid phosphatase components of the normal erythrocyte may be unequally affected by treatment with GSSG or APH. Only erythrocytes from normal subjects were used throughout the present series of experiments. Technical details have been reported elsewhere[3,4] and are briefly summarized in the legends to the figures.

Experiments on the incubation of hemolysates with GSSG. Hemolysates prepared from *A*, *B* or *CB* individuals and

[1] D. A. HOPKINSON, N. SPENCER, and H. HARRIS, Nature *199*, 969 (1963).
[2] The following abbreviations have been used: reduced glutathione = GSH; oxidized glutathione = GSSG; acetylphenylhydrazine = APH; red blood cells = RBC.
[3] E. BOTTINI and G. MODIANO, Biochem. biophys. Res. Commun. *17*, 260 (1964).
[4] E. BOTTINI and G. MODIANO, Experientia *21*, 379 (1965).

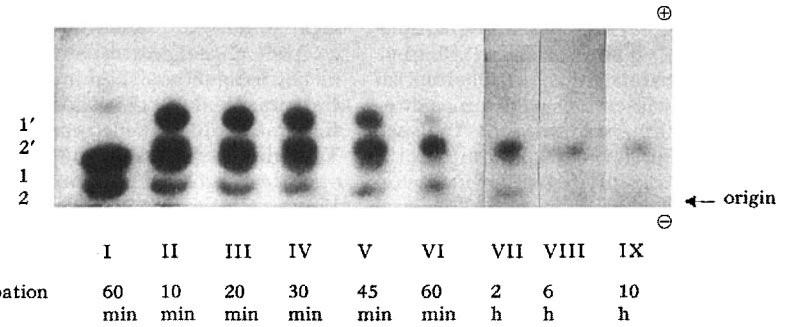


Fig. 1. Sequence of changes of the electrophoretic pattern of RBC acid phosphatases during incubation of hemolysates with GSSG. Slit I: Control sample: hemolysate of *B* type diluted 3:1 (v/v) with GSSG solvent and incubated at 37 °C for 60 min. Slits II–IX: Experimental samples: the same hemolysate of *B* type diluted 3:1 (v/v) with GSSG 0.065 *M* and incubated at 37 °C for the time indicated. Starch gel electrophoresis performed as previously described[3]. The duplication of the original electrophoretic pattern (slit I) is already evident within the first 10 min of incubation with GSSG (slits II–IV). If the incubation is prolonged, the fast component of the original pattern and its duplicated form start to fade off (slits V and VI) and they are no longer visible after 2 h of incubation (slit VII). The slower component of the original pattern and its duplicated form are instead detectable even after 10 h of incubation.

incubated with GSSG (see legend to Figure 1) showed a duplication of the electrophoretic pattern as if each component had been split in two and the new ones were faster than the original ones. The same effect of GSSG has been observed for the *CA* and *BA* phenotypes, though less clearly because of the higher complexity of the original pattern.

The changes induced by GSSG depend on the time of incubation, and they appear in the following sequence (see Figure 1): (1) the original pattern is doubled within the first 10 min of incubation; (2) the fast component of the original pattern and its duplication fade off after the first 2 h of incubation; (3) the slow component of the original pattern and its duplication fade off at much slower rate, both of them, but especially the faster one, being still detectable after 10 h of incubation.

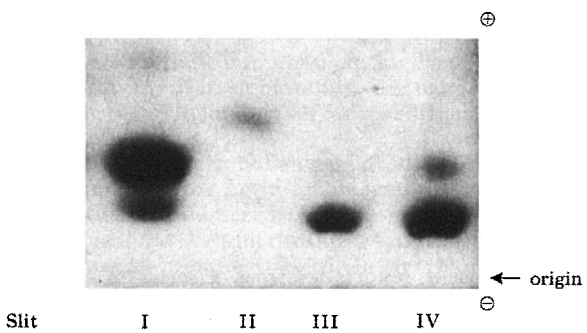


Fig. 2. Effect of GSSG and 2-mercaptoethanol on erythrocyte acid phosphatases. Slit I: Control sample: hemolysate (*B* type) diluted 3:1 (v/v) with solvent of GSSG and incubated 2 h at 37 °C. Slit II: Hemolysate treated with GSSG: the same hemolysate as in slit I diluted 3:1 (v/v) with GSSG 0.081 *M* and incubated 2 h at 37 °C. Slit III: Hemolysate treated with GSSG in the presence of 2-mercaptoethanol: the same hemolysate as in slit I diluted 6:1:1 (v/v/v) with GSSG 0.162 *M* and 2-mercaptoethanol 1.66 *M* and incubated 2 h at 37 °C. Slit IV: Hemolysate treated with GSSG and thereafter with 2-mercaptoethanol: the same hemolysate as in slit I was diluted 6:1 (v/v) with GSSG 0.162 *M* and incubated 2 h at 37 °C; at the end of 2nd h 2-mercaptoethanol 1.66 *M* (1 vol) was added and incubation continued for 1 h. Starch gel electrophoresis performed as previously described[3]. In slits I and II can be seen the original acid phosphatase pattern (type *B*) before and after treatment with GSSG. In slits III and IV it is evident that only the original acid phosphatase components are present though the fast ones are now weaker than the slowest, again suggesting a different liability to inactivation.

The suggested origin of bands 1′ and 2′ from 1 and 2 respectively (see Figure 1) is inferred by their relative distance and supported by additional evidence obtained with experiments of starch gel electrophoresis in 2 dimensions[5]. Thus it can be concluded that the treatment of the hemolysate with GSSG induces, besides the doubling of

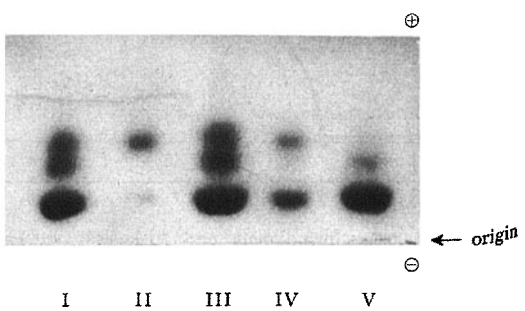


Fig. 3. Differential effect of APH on individual electrophoretic components of RBC acid phosphatases in the presence of glucose. Slit I: control sample, hemolysate of *CA* type diluted 3:1 (v/v) with GSSG solvent and incubated for 3 h at 37 °C. Slit II: hemolysate incubated for 3 h with GSSG 0.065 *M* as for the experiment described in Figure 1. Slit III: 2nd control sample, original hemolysate of *CA* type kept for 3 h at 4 °C. Slit IV: hemolysate from the same RBC pretreated with APH in the absence of glucose (RBC suspension 1:1 (v/v) in NaCl 0.9% incubated for 5 h at 37 °C with APH, 5 mg/ml). Slit V: the same as in slit IV but with added glucose (2% final concentration). Starch gel electrophoresis performed as previously described[3]. The Figure shows that the original acid phosphatase pattern (slits I and III) is altered in a very similar manner by incubation of the hemolysate with GSSG or incubation of RBC with APH in the absence of glucose (slit II and IV: note that the faster components seen in these slits are the duplicated forms of the slowest component of the *CA* original pattern). It shows, furthermore, that the addition of glucose to the RBC-APH incubation mixture prevents the duplication of the original pattern but does not prevent the fading of the faster components of the original pattern.

[5] These experiments were performed as follows: (a) electrophoresis of fresh hemolysates obtained from a *B* individual on starch gel according to the usual procedure; (b) incubation with GSSG of the relevant part of the gel containing the 2 normal components; (c) second electrophoretic run along a direction perpendicular to the first one of the separated and treated components transferred on a fresh gel.

the electrophoretic pattern, a progressive loss in enzyme activity which is more pronounced for the fast component of the original pattern and for its duplicated form.

The differential liability of the individual electrophoretic component of RBC acid phosphatases to the inactivation following treatment with GSSG, is further substantiated by a series of experiments with 2-mercaptoethanol. This reagent added to an hemolysate incubated with GSSG prevents or induces a reversion of the duplication of the original acid phosphatase pattern that GSSG produces when alone, but not of the fading of the faster component of the original pattern (see Figure 2)[6].

Experiments on the incubation of erythrocytes with APH. With a series of earlier experiments[4] we have shown that: (1) the incubation of erythrocytes with APH in the absence of glucose induces a modification of the electrophoretic pattern of RBC acid phosphatases very similar to the one obtained by incubating the hemolysates with GSSG; (2) no alteration of the electrophoretic pattern could be appreciated when the incubation was carried out in the presence of glucose.

However, we have later realized that the protective effect of glucose is not complete when the incubation is continued for a long time ($\pm$ 5 h). In this case, while the slower component of the original pattern remains unchanged, the faster one disappears almost completely, as is shown in Figure 3.

We feel that this phenomenon is of the same nature as the one observed in the experiments on the incubation of hemolysates with GSSG; that is to say, that the slower components of acid phosphatases are more stable than the faster ones towards an oxidative agent such as APH.

The results of these 2 series of experiments suggest a different liability of the acid phosphatase components to treatment with GSSG or APH; the slow fractions of the various phenotypes appearing as if they were more stable than the faster ones.

It is tantalizing to evaluate these observations against the quantitative and qualitative polymorphism of RBC acid phosphatases. One wonders whether there is any connection between the observed higher stability of the slower acid phosphatase components and the differences reported by SPENCER et al.[7] in the overall activity of the different acid phosphatase phenotypes.

From a more general standpoint, one wonders whether the differential liability of isoenzyme fractions towards toxic agents could result in a differential fitness in favour of the genotypes bearing the most stable combination of isoenzymes[8].

Riassunto. Le frazioni che costituiscono il normale quadro elettroforetico delle fosfatasi acide eritrocitarie mostrano una differente resistenza al trattamento con glutatione ossidato o con acetilfenilidrazina: le frazioni lente dei genotipi studiati sono apparse infatti più stabili.

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Gruppo di Ricerca «Commissione per la Genetica» del C.N.R., Istituto di Genetica e Clinica Pediatrica dell'Università di Roma (Italy),
September 5, 1966.

[6] A more detailed account on the protective effect of 2-mercaptoethanol on RBC acid phosphatases will be presented elsewhere.

[7] N. SPENCER, D. A. HOPKINSON, and H. HARRIS, Nature *201*, 299 (1964).

[8] Acknowledgment: We wish to express our gratitude to Prof. M. SINISCALCO and to Prof. D. CAVALLINI for their helpful criticism and for having read the manuscript before publication.

Monoaminergic Innervation of the Kidney. Aorticorenal Ganglion – A Sympathetic, Monoaminergic Ganglion Supplying the Renal Vessels

The aorticorenal ganglion is a small ganglion situated in the angle between the aorta and the renal artery. MAILLET[1] has described various lesions of the renal parenchyma after the chemical destruction of this ganglion. Afferent fibres run from the greater splanchnic nerve; efferent ones supply the renal plexus surrounding the renal artery (MITCHELL[2]). The ganglion sends filaments also to the mesenteric plexus. In the dog this ganglion is situated under the lower border of the suprarenal gland. After the removal of the left renal vein, we may find the ganglion when preparing the branches of the renal plexus which surround the renal artery. The ganglion was extirpated from 8 dogs on the left side and examined histologically. 6–8 days after the operation, the dogs were killed and the vegetative abdominal plexus of each animal prepared anatomically. In 6 cases the connection of the greater splanchnic nerve with the renal plexus was destroyed entirely; in the remaining 2 cases (dogs Nos. 4 and 6) the situation was different: in the vicinity of the examined ganglion a thin nerve branch was found which connected the mentioned vegetative nerves.

The left and right kidney of each dog were examined histologically. The silver impregnation techniques according to Bielschowski-Jabonero (after my own modification) and that of Bodian were used in all 8 cases. In 3 cases (dogs Nos. 6, 7 and 8) we also employed the histochemical fluorescence method according to FALCK[3]. A number of authors (FALCK[4], MALMFORS[5], and DAHLSTRÖM et al.[6]) have proved the high specificity of this method for catecholamines in ganglion cells in vegetative nerve terminals.

[1] M. MAILLET, Acta neuroveg. *20*, 337 (1960).

[2] G. A. G. MITCHELL, *Cardiovascular Innervation* (E. S. Livingstone Ltd., London 1956).

[3] B. FALCK and CH. OWMAN, Acta univ. lund., Sect. II, *7*, 1 (1965).

[4] B. FALCK, Acta physiol. scand., Suppl. 197, *56*, 1 (1962).

[5] T. MALMFORS, Acta physiol. scand., Suppl 246, *64*, 1 (1965).

[6] A. DAHLSTROM, K. FUXE, and N. Å. HILLARP, Acta pharmac. tox. *22*, 277 (1965).