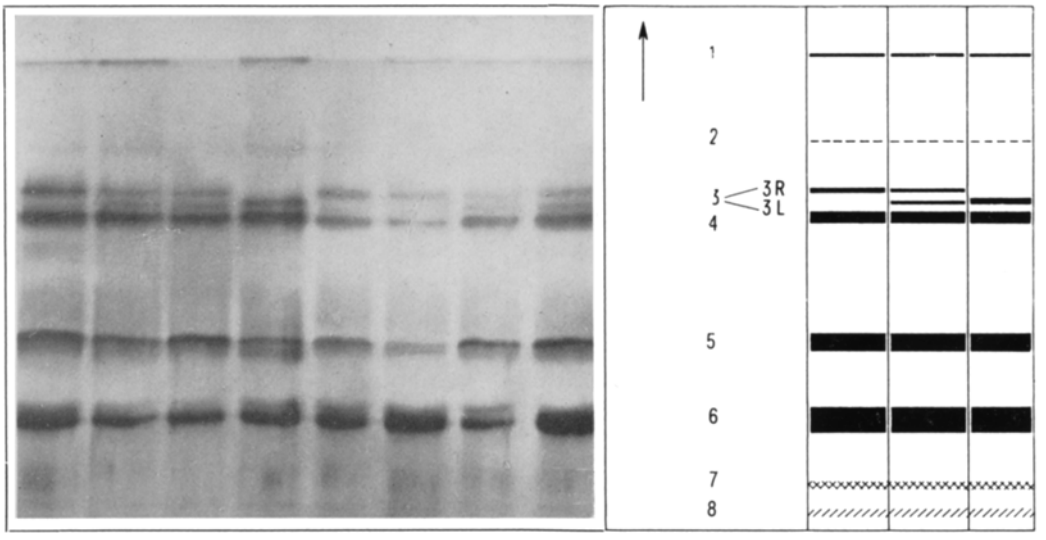

caractérisé par la présence de la bande 3R; le second par la présence de la bande 3L; le troisième possède ces 2 bandes. On remarque que la largeur des bandes 3R et 3L sur le protéinogramme du troisième type est plus réduite que celle des bandes de même mobilité des protéinogrammes des deux autres types.

Ces différences individuelles évoquent l'existence de 2 variants d'une protéine (protéine 3), sous le contrôle de 2 gènes allèles (gènes 3R et 3L).

La répartition des 3 types de protéinogrammes correspondant à des individus issus de 17 familles de frères et sœurs est indiquée dans le Tableau I.

Afin de vérifier cette hypothèse, nous avons procédé à des appariements entre sujets des familles 12 et 17 du tableau I. Les résultats et l'interprétation de ceux-ci figurent dans le Tableau II. La répartition des 3 types de protéinogrammes dans les 5 familles analysées est conforme à l'hypothèse proposée.

Discussion et conclusion. L'hémolymphe de *Galleria* est un fluide biologique complexe et instable, particulièrement sujet à mélanisation. La résolution de ses constituants dépend très largement des conditions expérimentales[3]. La technique que nous mettons en œuvre conduit à des résultats reproductibles; elle est suffisamment sensible pour permettre d'observer les modifications induites par la présence d'agents pathogènes[4,5] ou d'évaluer l'influence réelle de tout autre traitement sur la composition en protéine majeure de l'hémolymphe.

Cette étude met en évidence l'importance des variations héréditaires des protéines dans la définition du protéinogramme d'un insecte et constitue un des rares exemple de groupes sériques chez un invertébré.

Summary. The haemolymph proteinogram of *Galleria mellonella* larvae in the L3 stage is formed by 8 or 9 zones according to the larvae. The relative importance of various proteins shows only some slight individual differences. However, 3 distinct types of proteinogram relating to the presence or absence of near zones 3R and 3L are noted. The genetic analysis shows that these 3 phenotypes are induced by 2 codominant autosomal allelic genes. This work reveals the occurrence of blood groups in an experimental strain of Lepidoptera.

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The Effect of 2,3-DPG on Red Cell Enzymes

In 1971[1,2] we reported that several enzymes of glucose metabolism, viz. hexokinase, phosphoglucomutase, phosphofructokinase, aldolase and glyceraldehyde phosphate dehydrogenase were inhibited by 2,3-diphosphoglycerate (2,3-DPG). On the other hand, we found that glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, glucose-phosphate isomerase, triosephosphate isomerase, phosphoglycerate kinase, monophosphoglyceromutase, enolase, pyruvate kinase, glutathione reductase, and lactate dehydrogenase were not affected by 2,3-DPG.

Since our original report, most of our findings have been confirmed by others but some observations which may seem to contradict some of our conclusions, have been published. We should now like to explain these apparent discrepancies, and evaluate the possible physiologic role of some of the other points of inhibition which have been described.

The reports to which we refer include those of STAAL[3] claiming that phosphofructokinase is not inhibited by 2,3-DPG, YOSHIDA[4] reporting that 6-phosphogluconate dehydrogenase is inhibited by 2,3-DPG, ARNOLD et al.[5] who finds inhibition of glucose-phosphate isomerase and PONCE et al.[6] who discovered inhibition of phosphoglycerate kinase and pyruvate kinase in their studies.

The suggestion that purified PFK is not inhibited by 2,3-DPG is clearly in error. The results reported by STAAL and KOSTER are confused by the fact that they apparently used a 2,3-DPG preparation which was heavily contaminated with 3-PGA[7]. More important, however, is the fact that a very high concentration of *tris* was used in his assay system. We have found that such concentrations of *tris* completely relieve the inhibition of PFK by 2,3-DPG[8]. TARUI has confirmed that even highly purified phosphofructokinase is inhibited by 2,3-DPG[9].

We are able to substantiate YOSHIDA's report of the inhibition of 6-phosphogluconic dehydrogenase (6-PGD) by 2,3-DPG. In our original communication, no such inhibition was found because this enzyme was one of the few which was assayed at only one substrate concentration. The inhibition of this enzyme by 2,3-DPG is competitive with the substrate, 6-phosphogluconic acid (6-PGA). At the substrate concentration which we used, 0.6 mM, no inhibition is observed; at much lower, physiologic, concentrations, inhibition is quite apparent.

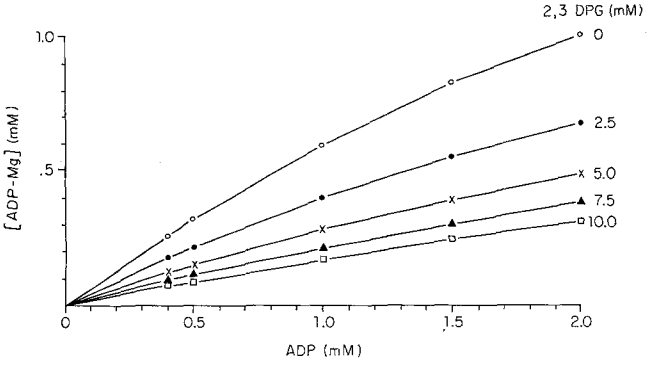

Fig. 1. The relationship between the total ADP concentration and that of the calculated ADP-magnesium complex in a system with a total magnesium concentration of 2 mM and 2,3-DPG concentrations varying from 0 to 10 mM. The concentration of the ADP-magnesium complex has been calculated from magnesium-2,3-DPG and magnesium-ADP dissociation constants of 10^{-3} M[10]. Similar curves are obtained with an ADP-magnesium complex dissociation constant of 3.03×10^{-4} M[12], but the effect of 2,3-DPG is then somewhat smaller.

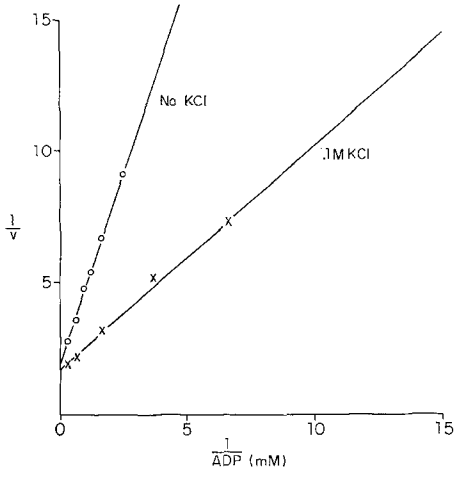

Fig. 2. A Lineweaver-Burke plot of pyruvate kinase activity at various levels of ADP. The assay was carried out as previously described[15]. In this system, the magnesium concentration is 10 mM, so that virtually all the ADP is complexed with magnesium. The K_m for ADP was 0.5 mM in the presence of 0.1 M potassium chloride; the K_m for ADP was 1.4 mM in the absence of potassium chloride.

We are able, too, to confirm the results reported by ARNOLD et al. Here, again, differences in technique account for the different results. The concentration of fructose-6-phosphate employed by ARNOLD et al. was only 50 μM, one-half the concentration we used. At this concentration of F-6-P slight inhibition of glucose-phosphate isomerase is seen also in our assay system. Secondly, the inhibition is more apparent at the pH of 7.2, used by ARNOLD et al., than at the pH of 8 which we employed. Finally, glucose-phosphate isomerase inhibition is partially overcome by the addition of magnesium which we employ in the assay while they do not. In any case, it seems unlikely that GPI inhibition by 2,3-DPG plays an important physiologic role, since, as pointed out by ARNOLD et al., only the backward reaction is affected.

The observations of PONCE et al. serve to emphasize the important role of the levels of magnesium-adenine nucleotide complexes in the regulation of kinases within the red cell. 2,3-DPG chelates magnesium with a dissociation constant of approximately 10^{-3} M[10,11]. The affinity of ADP for magnesium is relatively low, with dissociation constants having been estimated at approximately 10^{-3} M and 0.3×10^{-3} M[10,12]. Therefore, ADP-requiring reactions such as the pyruvate kinase (PK) reaction, are particularly sensitive to the free Mg concentration. The system which we use to assay PK contained 10 mM magnesium, while Ponce et al used a system containing only 2 mM magnesium ion. The calculated effect of 2,3-DPG on the concentration of the Mg-ADP complex is a system containing 2 mM Mg^{++} is shown in Figure 1. At the Mg^{++} and ADP concentration used by PONCE et al. the calculated ADP-Mg concentration is 0.81 mM. At a 5 mM concentration of 2,3-DPG the calculated ADP-Mg concentration falls to only 0.38 mM. In our system, because a great excess of Mg^{++} is present, the concentration of ADP-Mg falls only from 0.36 mM, in the absence of added 2,3-DPG, to 0.34 mM in the presence of 5 mM 2,3-DPG. Compounding the effect of 2,3-DPG in their magnesium-poor assay system is the fact that the system used by PONCE et al. contained no potassium. We have found that potassium markedly lowers the K_m of PK for ADP-Mg (Figure 2). These circumstances fully account for the fact that 2,3-DPG was found to inhibit pyruvate kinase in the assay system of PONCE et al. The effect was simply due to competition of 2,3-DPG for magnesium with ADP. A similar explanation of this discrepancy was recently offered by BLACK and HENDERSON[13]. The same type of effect on the phosphoglycerate

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kinase step can also be demonstrated. Indeed, PONCE et al. pointed out that 'inhibition' of these enzymes by 2,3-DPG was 'competitive' with Mg^{++}.

Although the effect effect of 2,3-DPG on these enzymes is merely due to Mg^{++} binding, this does not mean that it could not be physiologically significant. The total intracellular magnesium concentration of erythrocytes is only approximately 4.5 mM [14,15] and this small amount of magnesium is largely bound to ATP, a powerful magnesium chelating compound (dissociation constant $= 1.81 \times 10^{-5}$ M [12]) and 2,3-DPG. It is possible that, in spite of the high intracellular potassium concentration of red cells, 2,3-DPG may, under physiologic circumstances, produce some degree of modulation of magnesium requiring reactions [16].

Zusammenfassung. Der Einfluss von 2,3-DPG auf die Erythrozytenenzyme PFK, 6-PGD, GPI und PK ist nochmals untersucht worden. Unterschiede zu bereits publizierten Ergebnissen werden auf Grund unterschiedlicher experimenteller Bedingungen und verschiedener kinetischer Eigenschaften der Enzyme erklärt.

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Precipitating Antibody Against Core Glycolipid of *Enterobacteriaceae*

The cell walls of most *Enterobacteriaceae* possess a common 'core' glycolipid moiety which consists of 2-keto-3-deoxyoctonate (KDO) linked to lipid A [1,2]. Endotoxic properties of bacterial lipopolysaccharides have been related to the biologic activity of lipid A [3,4] and there is evidence that anti-KDO-lipid A antibodies are protective in man [5].

This report details methods for producing and demonstrating precipitating antibody against the core glycolipid moiety, as expressed in *S. minnesota* 595 chemotype 'Re'. The organism, whose cell wall consists principally of KDO-lipid A linkages [3,6], was obtained from Dr. OTTO WESTPHAL, Max Planck Institute for Immunobiologie, Freiburg (Germany). Albino New Zealand rabbits (2.5 to 4 kg), were immunized with suspensions containing 10^9 organisms/ml which were boiled for 1 h at 100 °C. Suspensions were injected i.v. according to the following schedule: 0.1 ml, 0.2 ml, 0.4 ml, 0.8 ml, 1.6 ml (followed by repetition of the last dose) at intervals of 5 to 7 days. Optimal antibody production was found in animals given more than 10 injections. Albino rabbits were also immunized with a 1 ml suspension of heat-killed organisms mixed with an equal volume of Freund's complete adjuvant (Difco Laboratories, Detroit, Michigan).

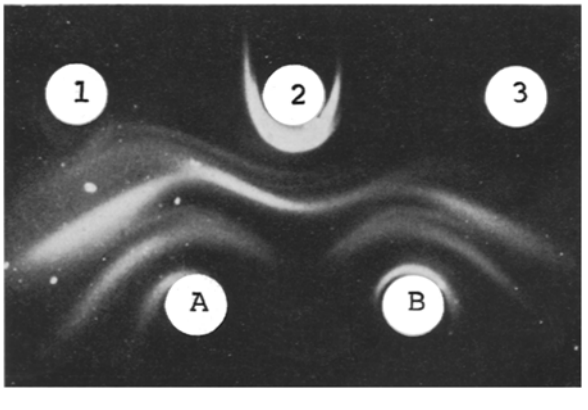

Fig. 1. Diffusion of *S. minnesota* 595 chemotype 'Re' antiserum against crude and purified antigens. A) Serum from rabbit immunized by i.v. route. B) Serum from rabbit immunized with heat-killed bacteria and Freund's complete adjuvant. Wells 1 and 3: Crude (freeze-thawed) antigen. Well 2: Purified antigen prepared by phenol-chloroform-petroleum ether extraction.

Lapine antibodies were raised against the following organisms by an initial i.m. injection of approximately 10^8 boiled bacteria followed by 4 to 6 i.v. injections of the same numbers of organisms every 5 to 7 days: *Escherichia coli* 014 (ATCC-19110), *Klebsiella pneumonia* type I, *Proteus rettgeri* type 80, and *Serratia marcescens* 01.

Crude antigenic preparations from all bacteria were prepared by methods identical to making the antigens used in immunizations except that they were additionally frozen at −80 °C and thawed 10 times. Antigen suspensions were centrifuged at 500 × g for 15 min and the supernatant used in immunodiffusion studies. Extraction of the glycolipid of *S. minnesota* 595 chemotype 'Re' was accomplished using the phenol-chloroform-petroleum ether method of GALANOS et al. [7] which yielded a water soluble antigen suspension containing 75 μg/ml of glycolipid.

The immunodiffusion method was that previously described using 1% agarose (Mann) thinly coated on glass slides [8]. Figure 1 demonstrates that serum from rabbits immunized by either method formed precipitin bands against the purified glycolipid preparation and crude *S. minnesota* antigen. In general sera from animals immunized by the i.v. route with 10 injections produced more intense bands of precipitation. A strong line of identity is observed between wells containing glycolipid extract and the crude supernatant antigen. A strong precipitin band close to the well containing the phenol chloroform-petroleum ether extract may have been due to antigen aggregation secondary to the extraction process. Figure 2 demonstrates precipitating antibody resulting

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