de liquide exuvial. Après dilution au 1/6 et centrifugation, les propriétés chitinolytiques de cette solution sont testées à différents pH (en tampon citrate-NaOH, conc. finale 0,1 *M*) (Fig.).

La cinétique de l'action chitinolytique du liquide exuvial est semblable à celle des autres chitinases connues (liquide intestinal de l'Escargot[1], exochitinase d'un Actinomycète isolé du sol[2]), qui manifestent également une activité optimale entre les pH 4,8 et 5,4. Par contre, nos résultats diffèrent nettement de ceux obtenus par HAMAMURA et al.[3] avec un extrait aqueux d'exuvies larvaires de *Bombyx mori;* le pH optimum pour la chitinase de cet extrait serait de 8,2, tandis que l'activité serait particulièrement faible au dessous du pH 6.

Nos observations confirment la présence d'une chitinase intervenant au cours du phénomène de la mue, hypothèse formulée notamment par WIGGLESWORTH[4]. La dissolution enzymatique de la chitine de l'endocuticule au moment de la mue avait été montrée par RENAUD[5] pour *Maia squinado* et par PASSONNEAU et WILLIAMS[6] pour *Platysamia cecropia.*

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Summary

The authors add further confirmation to the hypothesis of the role played by a chitinase during the process of moulting. By ligaturing *Bombyx mori*-larvae between head and thorax, accumulation of moulting fluid takes place between the larval and nymphal cuticles, swallowing of this fluid being prevented. The chitinolytic properties of this moulting fluid are demonstrated *in vitro.* The activity of chitinase at different pH is investigated: the optimum pH lying at about 5·4.

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On the Metabolic Significance of Blood Pyruvate in B_1 Avitaminosis

The researches of PETERS and coworkers[1] on the biochemical significance of Aneurin (cocarboxilase) have achieved a firmly established step forward in our knowledge of the mechanism of cellular oxidation. In consequence of their work it has been generally accepted that the hyperpyruvicaemia which occurs during B_1 avitaminosis is the direct expression of the delayed or interrupted oxidation of pyruvate. This opinion is supported by the *Catatorulin Effect in vitro* which was demonstrated by PETERS and coworkers. This phenomenon, which has been especially studied on the pigeons brain, is obtained only when the vitamin content of the tissue (*nervous*) has reached very low values. An analogous result[1] has recently been observed in the living organism of the rat in B_1 avitaminosis with regard to the metabolic response (*increase* of the R.Q. and heat production) after the administration of 1 g of glucose *per os.* This result is not obtained if the Vitamin B_1 content of the tissues is very low.

Since the vitamin B_1 content in the nervous tissue decreases more slowly than in other tissues[2], reaching values of about 0·5 μg per g only in very advanced stages of avitaminosis, it can be presumed that only in this stage, when there is a catatorulin effect *in vitro* and lack of a metabolic response *in vivo* to the administration of glucose, is the oxidative mechanism of the pyruvate sensibly altered (slowed or interrupted).

On the other hand, the blood pyruvate in the rat begins to increase after only 2 weeks of avitaminosis and the increase continues with increasing rapidity for another 2 weeks period during which the animal responds to the administration of glucose *per os* with an increase in the R.Q. and caloric production[3]. It does not seem probable in this period, in which the animal utilizes glucides in the same manner as the controls, that the alteration of the oxidation of pyruvate is such as to justify the already conspicuous increase in blood pyruvate.

The researches of DEANE and SHAW[4] and of SKELTON[5] have shown that the major part of the changes in the organs that result from B_1 avitaminosis in the rat (hypertrophy of the adrenals, involution of the thymus gland, renal hypertrophy, diminution of the ascorbic acid content of the adrenals, diminished secretion of gonadotropin and increased secretion of corticotropin by the prehypophysis) can be interpreted as a manifestation of the general syndrome of adaptation (SELYE). This syndrome is maintained by a hyperactivation of the hypophysis–adrenal system, as a consequence of fasting and the lack of vitamin B_1 in the diet. We are dealing therefore with aspecific symptoms, which are in common with other avitaminosis (B complex, pantothenic acid). Of all these alterations the most important is without doubt the hypertrophy of the adrenals, which was well demonstrated several years ago by MC CARRISON[6].

Recent researches by RINDI, FERRARI, and PERRI[7] have showed that the accumulation of pyruvate in the blood (rat) can be correlated statistically with the adrenal hypertrophy rather than with the values of the Vitamin B_1 content in the tissues.

To control experimentally whether and to what extent, the hypophysis-adrenal system influences the pyruvicaemia in B_1 avitaminosis, we followed the pyruvate variations in hypophysectomized and adrenalectomized rats. In the first case, 5 days after hypophysectomy

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[7] G. RINDI, G. FERRARI, and V. PERRI, Inter. Z. Vitaminforschg. *25*, 210 (1954).

the rats (100 g) were brought to a state of avitaminosis with the method previously described[1]. They were able to withstand the avitaminosis very well for more than 30–35 days. In the case of the adrenalectomized rats, with a shorter survival period, we had to use a different procedure: the rats were adrenalectomized at different phases of the avitaminosis and were then sacrificed 4–6 days after adrenalectomy.

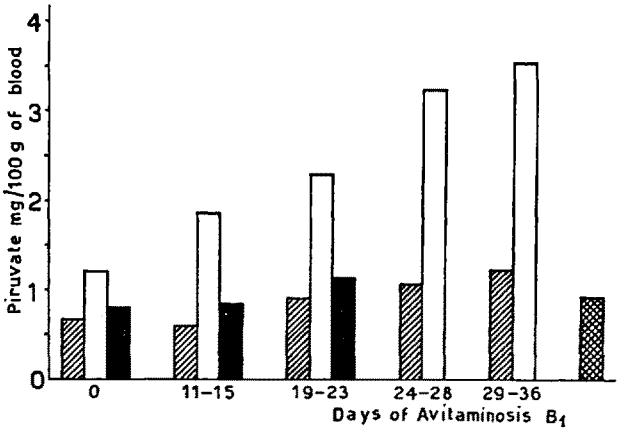

Fig. 1.–Blood Pyruvate of rats in Avitaminosis B_1: not operated, hypophysectomized and adrenalectomized.

□ Not operated rats in Avitaminosis B_1 (controls)

▨ Hypophysectomized rats in Avitaminosis B_1

■ Adrenalectomized rats in Avitaminosis B_1

▩ Hypophysectomized rats after 38 days (controls)

The results are shown synthetically in Figure 1. They show that in the hypophysectomized rats there is a B_1 avitaminosis hypopyruvicaemia and that adrenalectomy performed on rats in avitaminosis always causes a marked lowering of the pyruvicaemia. The course of the avitaminosis was followed by determining the Aneurin content of the tissues (brain, muscle, liver), which does not differ substantially from that of the controls. Even in normal rats hypophysectomy and adrenalectomy lower the pyruvate content of the blood.

Complete data will be published elsewhere[2].

From these results and from what is known on the increase of the pyruvicaemia in states of stress[3], or following the injection of cortisone or adrenal extracts in experimental animals[4], one might conclude that in B_1 avitaminosis the accumulation of pyruvate in the blood may be considered to a certain degree as due to the hyperfunction of the hypophysis-adrenal system.

These results do not exclude the possibility that part of the pyruvate that accumulates in the blood may be derived from a retarded or blocked oxidation of the pyruvate; but we have stated above the consideration that lead us to believe that the interruption or slowing down of the oxidative mechanism comes into play in the advanced stages of avitaminosis. The hyperpyruvicaemia which accompanies the B_1 avitaminosis is therefore a consequence of both hyperformation due to adrenal hyperfunction and to a slowed process of removal of the pyruvate, that is, it is in part an aspecific metabolic symptom, in part a specific symptom of the avitaminosis.

This interpretation permits us to explain a fact that would otherwise be difficult to explain: that is, the normal utilization of glucides by rats in avitaminosis from 3–4 weeks, when the blood pyruvate is already markedly increased[1].

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Riassunto

L'iperpiruvicemia che accompagna l'avitaminosi B_1 (ratto) è da considerarsi come dovuta, almeno in parte, all'iperfunzione del sistema ipofisi-surrene.

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Freisetzung von Kalium aus denerviertem und innerviertem Skelettmuskel durch Acetylcholin

In einer vorangegangenen Mitteilung konnten wir zeigen, dass nach intravenöser Verabreichung einer Reihe von neuromuskulär wirksamen quartären Ammoniumverbindungen (Succinylbischolin, Dekamethonium und Octamethylenbiscarbaminoylcholin), die eine Depolarisation der Endplattenregion bewirken, Kaliumionen aus der Muskulatur in die Extrazellularflüssigkeit in Freiheit gesetzt werden[1]. Die nachstehend mitgeteilten Versuche sollen zeigen, unter welchen Bedingungen und in welcher Grössenordnung eine Freisetzung von Kalium aus der Skelettmuskulatur durch Acetylcholin (ACh) erfolgt bzw. wie sich die Empfindlichkeitssteigerung des chronisch denervierten Muskels für ACh in dieser Hinsicht auswirkt.

Die Versuche wurden an Katzen mit isolierter Durchströmung beider hinterer Extremitäten in der gleichen Versuchsanordnung wie bei KLUPP und KRAUPP[1] beschrieben ausgeführt. Eine der beiden Extremitäten wurde dabei jeweils 8 bis 12 Tage vor dem Versuche durch Durchschneidung der N. ischiadicus und femoralis denerviert. Die durchströmten Extremitäten waren in einem Wasserbad von konstanter Temperatur von 37° versenkt. Als Durchströmungsflüssigkeit wurde eine Blut-Tyrode-Verdünnung (durchschnittliche Sauerstoffkapazität: 3,5 Vol%) verwendet. Die Kaliumbestimmungen wurden flammenphotometrisch durchgeführt.

Ergebnisse. In Vorversuchen wurde festgestellt, dass bei Durchflussgrössen von 10 bis 30 cm³ je Minute und Extremität die spontane Abgabe von Kalium an die Durchströmungsflüssigkeit den geringen Wert von 1 bis 2 µg/min auf der innervierten und von 2 bis 4 µg auf der denervierten Seite beträgt. In diesem Durchflussbereich blieb die arteriovenöse Konzentrationsdifferenz des Kaliums innerhalb des Versuches praktisch konstant, so dass die aus der Muskulatur an die Durchströmungsflüssigkeit abgegebenen Kaliummengen der Durchfluss-

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