amount of thromboplastin present, since about 15% of factor VII complex sufficed for maximal inactivation of undiluted thromboplastin under our experimental conditions.

Tab. I

Inactivating mixture		Clotting times after incubation of		
		1 min	30 min	60 min
Normal native serum 15%	Normal adsorbed serum 85%	12·6″	30·0″	34·1″
Reconstituted normal serum 15%	Normal adsorbed serum 85%	12·7″	29·0″	31·2″
Proconvertin deficient serum 15%	Normal adsorbed serum 85%	15·3″	16·5″	16·6″

Tab. II

Inactivating mixture		Clotting times after incubation of		
		1 min	30 min	60 min
Normal native serum 15%	Normal adsorbed serum 85%	12·6″	26·8″	33·9″
Reconstituted normal serum 20%	Normal adsorbed serum 80%	11·8″	27·4″	34·6″
Stuart factor deficient serum 20%	Normal adsorbed serum 80%	11·6″	26·2″	32·6″

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Résumé

Le rôle de la proconvertine et du facteur Stuart dans l'inactivation de la thromboplastine tissulaire par l'inhibiteur sérique a été examiné dans un système adéquat. Après avoir reconfirmé l'indispensabilité du complexe facteur VII pour l'action de l'inhibiteur, il a été constaté que seule la proconvertine est nécessaire pour l'inactivation tandis que le facteur Stuart ne paraît pas participer à ce processus.

Biological Properties of Synthetic Bradykinin

In the course of synthetic work on polypeptides BOISSONNAS, GUTTMANN, and JAQUENOUD recently synthesized[1] a nonapeptide with the following structure: H-L-Arg-L-Pro-L-Pro-Gly-L-Phe-L-Ser-L-Pro-L-Phe-L-Arg-OH.

A preliminary account of its biological activity was given in this journal[2] with the conclusion: 'Further work will demonstrate if it is identical with or closely similar to pure bradykinin'.

Active concentrations of different bradykinins on various biological structures.

	Synthetic bradykinin[5]	Natural bradykinin by the action of	
		Trypsin[6]	*Bothrops* venom[7]
Isolated guinea pig ileum	1 ng/ml	1 ng/ml	1 ng/ml
Isolated rat uterus	0.03 ng/ml	0.1 ng/ml	0.1 ng/ml
Blood pressure in the anaesthetized cat	500 ng/kg	400 ng/kg	30 ng/kg
Capillary permeability by intradermal injection in the guinea pig	1 ng	1 ng	10 ng

Since then ELLIOTT, LEWIS, and HORTON have, in view of the biological properties of this synthetic nonapeptide, reinvestigated their proposed structure for natural bradykinin[3] and, after new degradation studies, provided evidence[4] that natural bradykinin is a nonapeptide having the structure depicted above. Therefore, the previously reported synthetic nonapeptide[1, 2] is in fact synthetic bradykinin.

Further experimental work with this synthetic bradykinin by KONZETT and STÜRMER[5] has revealed that it behaves like pure natural bradykinin[6] in several tests. From a comparison of the quantitative data, some of which are given in the Table, it can be seen that the values for the activity of synthetic bradykinin and of natural pure bradykinin obtained from ox plasma by the action of trypsin are identical within the limits of biological variations. The conclusion therefore follows that, in its biological activity, synthetic bradykinin is identical with natural bradykinin obtained from ox plasma by the action of trypsin.

Data on the biological characteristics of pure natural bradykinin obtained from ox plasma by the action of *Bothrops jararaca* venom, which were reported by JAQUES and MEIER[7], have been included in the Table. They correspond reasonably well with the figures for the other natural bradykinin and for synthetic bradykinin. With regard to biological activity all three bradykinins behave similarly and cannot be distinguished from one another.

The synthesis of bradykinin opens up the possibility of using a pure and chemically defined polypeptide of high biological activity in medical research.

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Pharmakologisches und pharmazeutisch-chemisches Laboratorium der Sandoz AG., Basel, August 22, 1960.

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[2] R. A. BOISSONNAS, ST. GUTTMANN, J.-P. JAQUENOUD, H. KONZETT, and E. STÜRMER, Exper. *16*, 326 (1960).

[3] D. F. ELLIOTT, G. P. LEWIS, and E. W. HORTON, Biochem. J. *76*, 16 P (1960). These authors used throughout bradykinin obtained from ox plasma by the action of trypsin.

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[8] Present address: Pharmakologisches Institut der Universität Innsbruck (Österreich).

Zusammenfassung

Das synthetische Bradykinin H-L-Arg-L-Pro-L-Pro-Gly-L-Phe-L-Ser-L-Pro-L-Phe-L-Arg-OH hat (innerhalb der durch Unterschiede in der Methodik bedingten Fehlerbreite) die gleiche biologische Wirksamkeit wie natürliches, durch Einwirkung von Trypsin oder *Bothrops jararaca*-Gift aus Rinderplasma gewonnenes Bradykinin.

The Effect of Uncoupling Agents on Metabolism of Insect Muscle

It was stated by SACKTOR and CHANCE[1] that uncoupling agents are without effect on oxygen consumption of isolated mitochondria of insect muscle. We found, however, that 10^{-3} *M* 2,4-dinitrophenol has a pronounced effect on metabolism of the intact muscle of the American cockroach[2]. In denervated fibres, the effect was much smaller, which could not be accounted for by any changes in the activities of enzymes of different types. We will now present further data about the action of 2,4-dinitrophenol and another uncoupling agent dicumarol on muscles of *Periplaneta americana* L. and *Locusta migratoria* L.

The experiments were carried out on the intact muscle preparations described by GILMOUR[3] and KUBIŠTA[4]. The oxygen consumption was measured in Warburg respirometer and glycogen determined by anthron method. Both agents were prepared in physiological solution. The preparations were perfused with some drops of these solutions which were injected into the distal parts by means of a syringe. The oxygen consumption was measured for half an hour immediately on injection. In the case with high concentrations of 2,4-dinitrophenol, the oxygen consumption was highest at the beginning of the experiment and began to decrease after 10 min.

In anaerobic experiments, the uncoupling agents were injected 5 min after the beginning of the anaerobiosis; in this period, all oxygen is supposed to be removed.

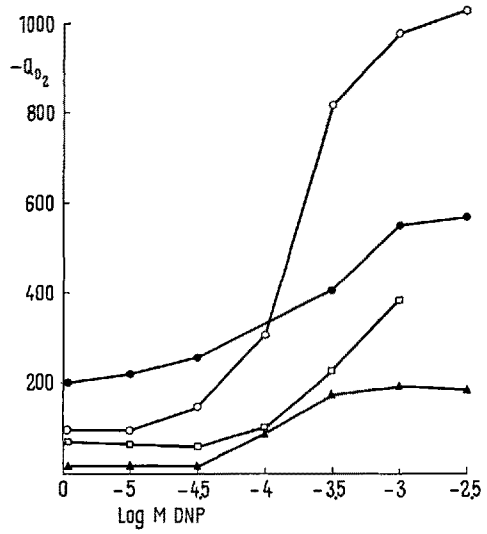
As may be seen from the Figure 2,4-dinitrophenol has a pronounced effect on the oxygen consumption of intact insect muscle preparations. The oxygen consumption is enhanced in both types of muscle fibres, i. e. in red muscles of the cockroach and also in white muscles of the locust which appear to have smaller resting values than cockroach muscles. The concentrations indicated in the Figure concern the injected solutions; the effective concentration within the muscle fibres was approximately calculated from the amount of 2,4-dinitrophenol determined analytically in the preparation and was found to be 50–60% of the concentration of the injected solution, provided that the dinitrophenol is equally distributed in the tissue.

24 days after nerve section, however, the effect of 2,4-dinitrophenol in the cockroach muscles is decreased. The differences of resting values between normal and denervated muscles are probably caused by the spontaneous activity observed in these muscles by BERÁNEK and NOVOTNÝ[5].

Dicumarol, another uncoupling agent, also caused an increase in oxygen conusmption, but its effect was smaller (Fig.) and no differences were observed between normal and denervated muscles as in the case of 2,4-dinitrophenol.

This effect of the uncoupling agents does not seem to be due solely to their action in the mechanism of oxidative phosphorylation, since both agents brought about an enhancement of metabolism in anaerobic condition (Table). The glycogen breakdown was increased particularly with 2,4-dinitrophenol. This corresponds to the findings of RONZONI and EHRENFEST[6] in frog muscle.

It appears, therefore, that the conditions for the action of uncoupling agents are more favorable in intact muscle fibres than in isolated mitochondria. Chronic denervation causes some unknown change in biochemical equipment of muscle which results in decreased sensitiveness to 2,4-dinitrophenol.



The effect of uncoupling agents on oxygen consumption. 2,4-Dinitrophenol: ○ = normal cockroach muscles; • = denervated cockroach muscles; ▾ = locust muscles; dicumarol: ▫ = cockroach muscles. Oxygen consumption expressed in 100 mmm³/g/h

The effect of uncoupling agents on anaerobic breakdown of glycogen in muscles of the cockroach. Temperature 22°C. Duration of anaerobiosis 20 min. Glycogen expressed in mg%

Uncoupling agent	Decrease of glycogen
None	172 ± 55
10^{-4} *M* 2,4-Dinitrophenol	276 ± 45
10^{-3} *M* 2,4-Dinitrophenol	346 ± 85
10^{-3} *M* Dicumarol	246 ± 39

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Zoological Institute of Charles University, Prague (Czechoslovakia), February 15, 1960.

Zusammenfassung

Der Einfluss von 2,4-Dinitrophenol und Dicumarol auf intakte Präparate von Insektenmuskeln wurde geprüft. Beide Stoffe, besonders aber 2,4-Dinitrophenol, riefen eine erhebliche Steigerung des aeroben und anaeroben Stoffwechsels hervor. Dauernde Denervation der Muskeln führt zu erniedrigter Empfindlichkeit gegen 2,4-Dinitrophenol.

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