

unknown in ants; intercastes are in nature rare, but easily produced experimentally, and are then intermediate in size as well as structure. If these facts are to be incorporated, the following hypothesis appears necessary: the duration of competence is brief and its onset dependent mainly on temperature; inductor concentration is proportional to size and has two significant thresholds, the lower initiating the development of queenness, and the higher completing it. To avoid natural intercastes and yet allow for their artificial production it is necessary to suppose that an extra impetus to growth is given to queen determined larvae and withheld or actually withdrawn from those which fail to secure induction. Intercastes would thus result from the metamorphosis of larvae with a concentration of inductor lying between the two thresholds.

Caste on this hypothesis would be determined as a result of a race between two processes, and it is encouraging to find, therefore, that in the related *Myrmica scabrinodis* temperature interferes differentially, for significantly more queens are produced in cultures at 18°C than at 25°C (in this respect these ants show a further resemblance to aphids). Failure of induction prevents additional increase in size and further differentiation. The worker is thus a form arrested before full potentiality is realised. Although it has no wing buds, it is not because they degenerate for they are extruded during metamorphosis, but are so small in their undeveloped state that they become merged with cuticular wrinkles by the time the adult form is reached (but are quite perceptible in the pupa). Degeneration does occur however in the ovariole rudiments.

So far, only the potentialities of hibernated larvae have been considered—and this accounts for half their full growth. The pre-dormancy factors relevant to caste, are not yet fully understood, but it seems that both dormancy and a period of "vernalisation" at low temperature are necessary for larvae to produce queens. Hibernation may thus be suitably construed as a period of quiescence in which the growth potentiality needed on regaining warmth, is built up. The size of larvae at dormancy is a function of nutrition in the preceding instars—not of egg-size, for this shows negligible variation. The fact that queens are only produced from hibernated larvae in *Myrmica* is part of a socio-ecological adaption which ensures that in the existing temperature-ontogeny relationship, sexuals are ripe for mating at the most suitable time of year. Queens are produced by large colonies in warm situations with abundant food supply, and in which the input of female brood is small in comparison with the vast labour force available to tend it.

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Zoological Department, Glasgow University, December 6, 1950.

Zusammenfassung

Bei Ameisen (*Genus Myrmica*) ist Kastenbestimmung rein trophisch. Königindetermination hängt davon ab, daß die weiblichen Larven vor einer bestimmten Zeit eine gewisse Größe erreichen und einen zusätzlichen Wachstumsanstoss zur Folge haben. Dieser Mechanismus erinnert an die für die Aphiden beschriebenen Vorgänge.

Biosynthesis of Cholesterol from Isobutyrate¹

The utilization of the branched chain of isobutyric acid by the intact rat has been described in a previous

paper¹. At that time, the radioactivity incorporated into the various liver fractions was reported. It has now been demonstrated that an appreciable amount of radioactivity is incorporated into the carcass cholesterol and carcass fatty acid. In feeding experiments with deuterium-labeled isobutyrate, RITTENBERG and SCHOENHEIMER² and BLOCH³ reported that little or no deuterium was incorporated into cholesterol.

The method of injection and other experimental details have already been described¹. After sacrifice of the rat, the carcass was homogenized and dried by lyophilization. The dried carcass was extracted with ether-alcohol 1:3 for 100 hours and the fatty acid and nonsaponifiable fractions were separated in the usual manner.

In the first case a total of 19.6 μ C of isobutyrate were injected. Cholesterol, m. p. 147–148°, was isolated through the digitonide. The infra red spectrum of the extracted cholesterol was identical with that of an authentic sample⁴. In chromatography on "Quilon" treated paper⁵, the cholesterol showed an R_f of 0.55 when methanol was the developing solvent. A radioautograph⁶ showed that no other radioactive material was present.

The specific activity of the cholesterol was found to be 1500 disintegrations/min/mg C⁷. The specific activity of the carcass fatty acids was 1000 dis/min/mg C.

In two similar experiments in which 19.6 μ C and 40.5 μ C of isobutyrate were injected, cholesterol was isolated as the digitonide and assayed for radioactivity in this form. The specific activities, calculated for cholesterol, were 800 dis/min/mg C and 1800 dis/min/mg C respectively.

Acknowledgment: – The interest shown by Dr. MELVIN CALVIN during the course of this work is gratefully acknowledged.

DAVID KRITCHEVSKY and IRVING GRAY⁸

Radiation Laboratory, University of California, Berkeley, February 9, 1951.

Zusammenfassung

Es wird der Beweis erbracht, daß die Methyl-Gruppen der Isobuttersäure in das Cholesterin der Ratte einverleibt werden.

¹ I. Gray, P. ADAMS, and H. HAUPTMANN, *Exper.* **6**, 430 (1950).

² D. RITTENBERG and R. SCHOENHEIMER, *J. Biol. Chem.* **121**, 235 (1937).

³ K. BLOCH, *J. Biol. Chem.* **155**, 255 (1944).

⁴ We are indebted to Dr. N. K. FREEMAN and Mr. YOOK NG OF DONNER Laboratory for determination of these spectra.

⁵ D. KRITCHEVSKY and M. CALVIN, *J. Amer. Chem. Soc.* **72**, 4330 (1950).

⁶ A. A. BENSON, *et al.*, *J. Amer. Chem. Soc.* **72**, 1710 (1950).

⁷ All measurements of radioactivity on "Nucleometer" windowless counter.

⁸ Major, Medical Service Corps, U. S. Army. Present address: Brooke Army Medical Center, Fort Sam Houston, Texas.

Zur Bausteinanalyse des Clupeins

In ihren letzten Mitteilungen «Über Clupein» haben K. FELIX und Mitarbeiter¹ über die Feststellung berichtet, daß in dem von ihnen untersuchten Präparat von Clupeinmethylester-hydrochlorid neben Arginin, Alanin, Valin, Serin und Prolin als den schon bekannten Haupt-

¹ The work described in this paper was sponsored by the U. S. Atomic Energy Commission.

¹ K. FELIX, H. FISCHER, A. KREKELS und H. M. RAUEN, *Z. physiol. Chem.* **286**, 67 (1950). – K. FELIX, H. M. RAUEN, W. STAMM und G. ZIMMER, *ebenda* **286**, 199 (1950).

bausteinen¹ mit der Methode der Papierchromatographie noch Threonin und Isoleucin, wenn auch in geringeren Mengen, als Bausteine nachgewiesen und daß weiterhin nach Einwirkung von Dinitrofluorbenzol auf das untersuchte Clupeinpräparat sowohl Prolin wie Serin als amino-endständige Bausteine gekennzeichnet werden konnten.

Die Untersuchung eines von uns aus dem Rohclupein durch Umscheidung aus konzentrierter wässriger Lösung in der Kälte bis zu konstanter Höhe des Quotienten $N:NH_2 = 125$ gereinigten Clupeinpräparates, das den Hauptanteil (etwa 70%) des Rohclupeins darstellt², ergibt demgegenüber die folgenden Befunde:

1. Das Präparat enthält zufolge der papierchromatographischen Analyse neben Arginin nur die 4 Monoaminosäuren Serin, Valin, Alanin und Prolin, und zwar die letzteren in dem schon früher³ festgestellten molekularen Verhältnis von 2:1:1:1; weder Isoleucin noch Threonin sind darin auch nur in Spuren nachweisbar.

2. Die Analyse der Spaltprodukte nach Einführung des Dinitrophenylrestes in unser Clupein ergibt als einzige substituierte Aminosäure Prolin; neben dem allein gebildeten Dinitrophenylprolin ist auch papierchromatographisch kein Dinitrophenylserin nachzuweisen. Prolin stellt also in unserem Präparat die einzige amino-endständige Aminosäure dar.

Aus diesen Befunden insgesamt ergeben sich die folgenden Schlußfolgerungen:

Durch die von uns vorgenommene Fraktionierung des Rohclupeins auf Grund des Gehaltes an Aminostickstoff gelingt es, die Hauptkomponente desselben, mit endständigem Prolin, die ja durch den niedrigsten Gehalt an Aminostickstoff ausgezeichnet ist, frei von den anderen Komponenten des ursprünglichen Mischstoffs⁴ zu erhalten. Das von FELIX und Mitarbeitern untersuchte, nach einem anderen Verfahren fraktionierte Clupeinpräparat stellt danach offenbar noch ein Gemisch zweier Clupeinanteile dar, deren einer Prolin und deren zweiter Serin als endständige Aminosäure trägt; Isoleucin und Threonin scheinen Bausteine der serin-endständigen Komponente zu sein.

Für die Kennzeichnung der Natur der Bausteine und ihrer Reihenfolge im Molekül, die angestrebt wird, sollten daher nur solche Präparate gewählt werden, die zum Beispiel zufolge ihrer Baustein- und ihrer Endbausteinanalyse sich nicht als Mischstoffe erweisen.

E. WALDSCHMIDT-LEITZ, K. KÜHN und FR. ZINNERT
München, den 17. Februar 1951.

Summary

It has been found that clupein, purified by a fractionation of its aqueous solution in the cold, contains only arginine, serine, alanine, valine and proline as constituents, and that a proline residue is placed at the amino end of the peptide chain of its molecule.

¹ Vgl. A. KOSSEL, *Protamine und Histone* (Fr. Deuticke, Leipzig und Wien, 1929). – E. WALDSCHMIDT-LEITZ und E. GÜNTHER, *Makromolekulare Chemie* 2, 120 (1947/48).

² Vgl. E. WALDSCHMIDT-LEITZ, F. ZIEGLER, A. SCHÄFFNER und L. WEIL, *Z. physiol. Chem.* 197, 219, und zwar S. 224ff. (1931).

³ E. WALDSCHMIDT-LEITZ und E. GÜNTHER, *l. c.*

⁴ Gemäß der Definition von H. STAUDINGER, *Makromolekulare Chemie und Biologie* (Basel, 1947, S. 126).

Similar Effects of Methylene Blue and of Vitamin E on Liver Storage of Vitamin A in Chicks

As will be reported elsewhere¹, certain manifestations of vitamin-E deficiency in chicks and rats can be prevented by dietary supplements of some substances which easily undergo reduction-oxidation in the body. This applies to the exudative diathesis, encephalomalacia in chicks, and peroxidation and yellow-brown discoloration of adipose tissue in chicks and rats, as well as to depigmentation of the incisors in rats. All these conditions, which are caused by feeding certain highly unsaturated fatty acids, such as those in cod liver oil together with vitamin-E deficient diets, can be prevented by dietary supplements of vitamin E, for instance in the form of *d,l*- α -tocopherol acetate, and greatly depressed by dietary additions of methylene blue, thionine, thiodiphenylamine and several similar compounds. Along with the effect on the symptoms, in chicks, a considerable improvement in growth rate is observed.

In order to study further the cause of the improved growth rate we determined (by the Carr-Price reaction as well as by measurement of the absorption at 325 m μ) the deposition of vitamin A in the liver of chicks receiving an exudate producing diet with 10% cod liver oil, with and without supplements of *d,l*- α -tocopherol acetate (10 mg%) and methylene blue (126 mg%). The determinations were carried out when the chicks had been on the experimental diet for 4 weeks from the 10th day after hatching. They showed that the feeding of methylene blue resulted in a very considerable increase in vitamin A storage in the liver, indicating that also in this respect methylene blue exerts an effect known to be exhibited by vitamin E.

The average values for vitamin A per gram fresh liver, standard deviation of the mean, number of animals and average weight were as follows:

Diet	Vitamin A i. u. per g liver	No. of chicks	Weight g
Basal			
E-deficient, no. 536, DAM et al. ²	68 $\pm$ 13	6	161
Basal + 10 mg% <i>d,l</i> - α -tocopherol acetate	386 $\pm$ 47	9	226
Basal + 126 mg% methylene blue	903 $\pm$ 106	10	252

A full account of the experiment will be published elsewhere.

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HENRIK DAM, INGE PRANGE, and EBBE SØNDERGAARD

Department of Biology, Polytechnic Institute, Copenhagen, November 24, 1950.

Zusammenfassung

Küken, die mit einer Dorschlebertran enthaltenden, vitamin-E-freien Nahrung gefüttert werden, lagern viel mehr Vitamin A in der Leber ab, wenn die Nahrung auch Methylenblau enthält. Diese Wirkung des Methylenblaus auf die Vitamin-A-Speicherung ähnelt dem entsprechenden Effekt von Vitamin E und beruht wahrscheinlich auf einer antioxydierenden Wirkung des Leukomethylenblaus.

¹ H. DAM, I. KRUSE, I. PRANGE, and E. SØNDERGAARD, *Acta Physiol. Scand.* (in press). – H. DAM and H. GRANADOS, *Acta Physiol. Scand.* (in press).

² H. DAM, I. KRUSE, I. PRANGE, and E. SØNDERGAARD, *Acta Physiol. Scand.* (in press).