der Reinigung von Lipoid A ausgeschlossen ist. Die quantitativen Unterschiede können nicht auf einen wechselnden Lipoidgehalt der einzelnen Präparate zurückgeführt werden, sondern sind offenbar durch die physikalisch-chemischen Eigenschaften der Trägersubstanzen mitbedingt. Polysaccharide als lyophile Träger scheinen in dieser Beziehung besonders günstig, wie wir auch am Beispiel anderer biologischer Wirkungen (Pyrogenität u.a.) zeigen konnten.

Die durchweg etwas geringere Wirksamkeit der Präparate aus *S. abortus equi* gegenüber denjenigen aus *E. coli* 08 stimmt mit unserer Erfahrung überein, wonach das Lipoid A aus *S. abortus equi* gegen Hydrolyse und andere chemische Einflüsse weniger stabil ist als dasjenige aus *E. coli*.

Der Polysaccharidanteil in den bakteriellen Lipopolysacchariden vermittelt die Wasserlöslichkeit der Präparate und damit den hohen Dispersionszustand des Lipoids A in Lösung; das Polysaccharid ist überdies Träger der immunspezifischen Eigenschaften (0-Antigen) der betreffenden Bakterienart[13]. Demgegenüber enthält der Lipoid-Anteil (Lipoid A) die für die endotoxischen Wirkungen wesentlichen Wirkgruppen[14]. Auf Grund der vorliegenden Versuche über die leukotaktische Wirkung der bakteriellen Lipopolysaccharide kann geschlossen werden, *dass hierfür ebenfalls Wirkgruppen im jeweiligen Lipoid-A-Anteil wesentlich sind.*

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Summary

Highly purified lipopolysaccharides derived from gram-negative bacteria (endotoxins) stimulate the migration of leucocytes from cultures of chick leucocytes. This effect, as well as many endotoxic manifestations of the preparations *in vivo* (pyrogenicity, toxicity, etc.), is induced by their lipid fraction (lipid A), while the respective species-specific polysaccharide functions as lyophilizing carrier which, fundamentally, can be substituted by other inert carriers (e.g. protein).

[13] O. WESTPHAL und O. LÜDERITZ, Angew. Chemie *66*, 407 (1954). – Siehe zum Beispiel A. M. STAUB und R. TINELLI, Bull. Soc. Chim. biol., Paris *39*, Suppl. I, 65 (1957).

[14] E. NETER, O. WESTPHAL, O. LÜDERITZ, E. A. GORZYNSKI und E. EICHENBERGER, J. Immunol. *76*, 377 (1956). – O. WESTPHAL, O. LÜDERITZ, E. EICHENBERGER und E. NETER in *Chemistry and Biology of Mucopolysaccharides*, Ciba Found. Symp. (J. & A. Churchill Ltd., London 1958), p. 187.

Exudative Diathesis Produced by Vitamin E-Deficient Diets Without Polyenoic Fatty Acids

In earlier studies (DAM, KOFOED NIELSEN, PRANGE, and SØNDERGAARD[1]) we have examined the content of polyenoic fatty acids in two kinds of yeast, viz. Torula 3N and Fleischmann 50B, used as the source of protein in vitamin E-deficient diets for chicks. Torula 3N, which gave rise to exudates, contained 5·3% total fatty acids with 44·7% dienoic and 2·6% trienoic, whereas Fleischmann 50B, which did not produce exudates, contained almost no polyenoic fatty acids. Torula yeast extracted with alcohol still contained 2·7% total fatty acids with 45·3% dienoic and 3·6% trienoic[1], and gave rise to exudates (BIERI, POLLARD, and BRIGGS[2]).

Therefore, in analogy with previous experiences with casein-cod liver oil diets, it seems reasonable to assume that, also in the experiments with Torula yeast, the polyenoic fatty acids are an important factor in the development of exudative diathesis.

In a search for possible additional factors, we have examined a series of different yeasts with respect to their fat content and ability to produce exudative diathesis in chicks. Among these yeasts we have found one which is of particular interest, because, as in Fleischmann yeast 50B, the content of polyenoic fatty acids is practically nil, and nevertheless it produces exudates in chicks when fed without vitamin E. This yeast is a dried *Saccharomyces cerevisiae* obtained from The Distillers Company Ltd., London under the designation 'Flake Yeast'. It contained 4·4% total fatty acids and 2·0% unsaponifiable matter. The content of dienoic fatty acids in the total fatty acids was 0·6%. The amounts of trienoic, tetraenoic, pentaenoic, and hexaenoic acids were nil. No significant amount of trans fatty acids could be detected by means of infrared spectrophotometry.

Composition of basal diets, g per 100 g

Flake yeast	40·00	60·00
Gelatine	3·00	3·00
Salt mixture[3]	5·17	5·17
Vitamin mixture[3]	0·10	0·10
Choline chloride	0·20	0·20
Sucrose	51·53	31·53

Plus 0·001 g dicalcium salt of 2-methyl-1,4-naphthohydroquinone diphosphoric acid ester (Synkavit 'Roche') per 100 g diet.

Vitamins A and D_3 were given in aqueous solution[4], 0·1 ml twice a week.

When fed at a 40%, resp. 60% level in a diet of the composition indicated in the Table, 'Flake yeast' caused exudates in 9, resp. 5 out of 10 chicks within 29 days. In a repetition of the experiment, 8, resp. 6 out of 10 chicks showed exudates within 33 days. These results suggest that 'Flake yeast' contains an insufficient amount of a protective factor. The exudates were less severe than those produced by corresponding Torula yeast diets.

One more peculiarity was noted in the feeding experiments with 'Flake yeast'. Fat tissue in which exudate had occurred contained, in 19 out of 23 cases, no peroxide detectable by the method of KING, ROSCHEN, and IRWIN[5], modified by DAM and GRANADOS[6].

This might indicate that the *in vivo* peroxidation of body fat usually observed in connection with exudative diathesis produced by casein-cod liver oil diets and (to a

[1] H. DAM, G. KOFOED NIELSEN, I. PRANGE, and E. SØNDERGAARD, Exper. *13*, 493 (1957).

[2] J. G. BIERI, C. J. POLLARD, and G. M. BRIGGS, Fed. Proc. *16*, 381 (1957).

[3] H. DAM and E. SØNDERGAARD, Acta pharm. tox., Kbh. *9*, 131 (1953).

[4] H. DAM, S. HARTMANN, J. E. JACOBSEN, and E. SØNDERGAARD, Acta physiol. scand. *41*, 149 (1957), Table 2.

[5] A. E. KING, H. L. ROSCHEN, and W. H. IRWIN, Oil & Soap *10*, 105 (1933).

[6] H. DAM and H. GRANADOS, Acta physiol. scand. *10*, 162 (1945).

lesser degree) by Torula yeast diets is not *per se* the cause of the exudation process but a sign of the disappearance of the antioxidant vitamin E from tissue simultaneously containing a certain amount of polyenoic fatty acids. Further, it is possible that the hematin compounds released in the hemorrhagic phase of the exudation process accelerate an existing tendency to peroxidation (cf. TAPPEL[7]).

These considerations may also serve to explain the observation that selenium dioxide prevents peroxidation in chicks fed vitamin E-free Torula yeast diets: by preventing exudates, selenium will slow down the appearance of peroxides otherwise accelerated through the hemorrhagic phase of the exudation process.

Selenium dioxide does not counteract the *in vitro* autoxidation of cod liver oil incorporated in casein diets, nor does it prevent brown coloration and peroxidation of depot fat in rats fed vitamin E-free cod liver oil-casein diets[8]. It is therefore unlikely that the protective action of selenium dioxide against the exudative diathesis in chicks is due to an *in vivo* antioxidant effect.

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Zusammenfassung

Bei Fütterung von Küken mit einer Vitamin-E-freien Nahrung, die als Eiweissquelle eine spezielle Trockenhefe, frei von mehrfach ungesättigten Fettsäuren, enthielt, wurde eine jedoch verhältnismässig milde, exsudative Diathese beobachtet.

[7] A. L. TAPPEL, Arch. Biochem. Biophys. *44*, 378 (1953); J. biol. Chem. *217*, 721 (1955).

[8] Paper in preparation.

Isolation of a Toxic Fraction from Uraemic Blood

In our former studies[1], it was observed that uraemic blood streaming through Dovex 50, Dovex 2, and IR 4B ion-exchange resins would become toxic. The fact that similarly treated control blood samples did not become toxic led us to the assumption that uraemic blood might contain some preformed toxin.

In the present experiment, we aimed at producing this substance in a more concentrated form.

Methods. Dogs were subjected to bilateral nephrectomy under sterile conditions. 72–96 h postoperatively the animals were exsanguinated and the cerebrospinal fluid was obtained through cisternal puncture. The toxicity tests were performed on rats and mice.

15 nephrectomized and 8 control dogs and 35 human blood specimens (uraemic or of diseases of non-renal origin), as well as the cerebrospinal fluid of 14 human subjects and of 5 dogs, were used in the course of the experiment.

Experimental. For purifying the toxin, the acid alcoholic fraction has so far proved to be the most effective.

[1] I. DÉSI, I. FEHÉR, P. WEISZ, and E. SZOLD, Z. ges. inn. Med. *24*, 1127 (1957).

Kind of experiment	Number of experiments	Average of NPN mg%	Number of animals used	Number of animals that died
Uraemic blood . .	28	99 (53·5–195)	mice 19 rats 63	17 58
Control blood . .	30	36 (28·8–48)	mice 17 rats 27	0 0
Uraemic cerebrospinal fluid . . .	5	150 (128–174)	rats 7	7
Control cerebrospinal fluid . . .	14	20 (6–42)	rats 14	0

100 ml of plasma centrifuged until pure were dialyzed at a temperature of about + 5°C in cellophane bag in flowing water. Aethyl alcohol was added until a 50% concentration and acetic until a 1% final concentration was achieved. The dense substance full of precipitation was then placed in a hot-water bath for 10 min and centrifuged. The deposit was twice washed in 25 ml of 75% alcohol and the precipitation was discarded. The washing fluid and the supernatant were mixed and condensed in vacuum from the water bath to $^1/_6$ of the original volume. 0·08 g of $NaHCO_3$ and 50 ml of 70% alcohol were added to the residue, whereafter the substance was repeatedly centrifuged and washed as before. The mixed fluids were then evaporated from water bath to achieve the $^1/_{10}$ of the original volume. The evaporation was performed in vacuum. The substance was shaken out with a small quantity of charcoal (50–80 mg/10 ml of the substance) and the sediment was cast off. The substance thus obtained was used in the further experiments.

The K, Na, Cl, P, protein- and non-protein nitrogen of preparates thus obtained, showed no difference whether originating from the serum of uraemic or control dogs. According to the administered dose, the toxin caused various symptoms in 50–80 g rats. The toxin was injected intraperitoneally. 1–1·5 ml of the substance would bring about death of the animals with violent tonic spasms and dyspnoe within 30 min. We suppose respiratory paralysis to be the immediate cause of death. The administration of a smaller dose killed the animals within 5 h, while a still smaller dose resulted in death within 24 h. The administration of a medium dose produced the first symptoms after 15–30 min. The spontaneous activity of the animal was reduced and later on disappeared. First there is a response to the sensation of pain, the animal trying to run away, but the movements are incoordinated, the gait is unsteady with a tendency to fall; when it tries to run again, it rolls about a few times and falls again after a few steps. Exhaustion takes place very soon and the animal shows no response to repeated stimuli, becoming completely atonic after a short while. There is no more sensation of pain, it remains still and completely atonic. Respiration becomes shallow and overhasty and the whole condition is interrupted by paroxisms of tonic convulsions in the midst of which the animal dies (Table).

The blood pressure of the intoxicated animals was taken in 10 cases, but no reduction was shown even in the end state.

A correlation was observed between the amount of toxin injected and the variations in the rectal temperature of the rats. The measurements were carried out under conditions corresponding to 18–20°C room temperature. There was a 10° reduction in the temperature within 2 h due to large doses. There was a 3°C reduction of temperature also after the administration of control plasma, but