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<br>rats 63 | 17<br>58 |
| Control blood . . | 30 | 36 (28·8–48) | mice 17<br>rats 27 | 0<br>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.

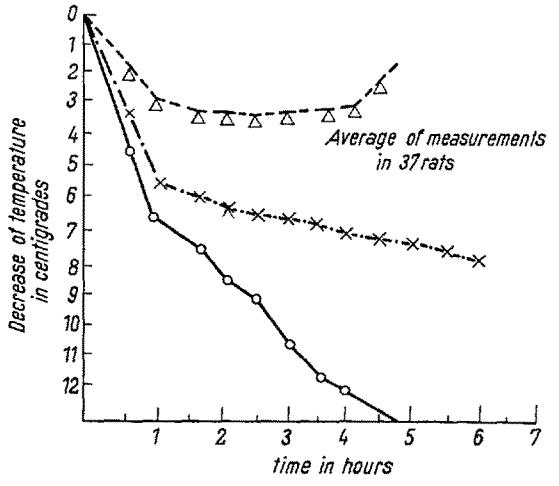
The K, Na, Cl, P, protein- and non-protein nitrogen of preparates thus obtained, showed no difference whether originating from the serum of uraemic or control dogs. According to the administered dose, the toxin caused various symptoms in 50–80 g rats. The toxin was injected intraperitoneally. 1–1·5 ml 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

a few hours later the animals regained their original temperature and never died (Figure).

Changes in the rectal temperature of rats after injecting uraemic and control preparates




Key to the signs:

○—○—○ average of animals, died in 5 h
×—×—× average of animals, died in 24 h
△—△—△ average of control animals

Toxin obtained from the blood and cerebrospinal fluid of uraemic patients produced the same symptoms. Preparates obtained from healthy individuals, or of others suffering from any disease of non-renal origin, failed to produce these symptoms.

Since it is wellknown that hypothermy is one of the clinical symptoms of human uraemia[2], we tested whether this phenomenon was demonstrable in animals as well. After bilateral nephrectomy of 45 rats, we found that the temperature showed a gradual reduction between the operation and the death of the animals, reaching a level of about 25°C.

The preparate will become inactive if dyalized in flowing water in a cellophane bag suggesting that the toxin is a substance of small molecules. Since the native uraemic plasma has no toxic effect, but would become effective if dialyzed and prepared in the manner described above, we concluded that the toxin may circulate in the uraemic blood in the form bound to protein or to some other large molecule without being active in that state.

Incubated with pancreatic suspension or trypsine (Merck), the toxin would become inactive, suggesting that our substance may be a peptide.

Since, as a rule, the animals died after a severe drop in temperature, we tried to avert the action of toxin by the administration of a pyrogen agent. 1 h after receiving the toxin, the animals were injected intraperitoneally with 20 mg/kg body weight of Amphetamine. After this dose, the reduction in temperature discontinued, but the animals still died, even earlier than those treated only with the toxin.

Summarizing the results, it may be stated that we succeeded in demonstrating from blood samples of uraemic individuals and dogs, and from their cerebrospinal fluid, a substance as yet unknown as far as we know, which might play a role in the production of uraemic symptoms. Judging by the experiments carried out so far, the substance is considered to be a peptide of small molecules.

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*Zusammenfassung*

Aus urämischem Menschen- und Hundeblut und Liquor isolierten wir eine uns bisher unbekannte Substanz, die unserer Ansicht nach für die Entstehung von urämischen Symptomen mitverantwortlich sein dürfte. Auf Grund unserer bisherigen Untersuchungen halten wir die Substanz für ein kleinmolekulares Peptid.

## Acetylcholinesterase, Acid Phosphatase, and Succinic Dehydrogenase in the Hypothalamic Magnocellular Nuclei after Chlorpromazine Administration

*Histochemical studies*

Strong enzyme activity has been found to exist in the magnocellular nuclei of the hypothalamus, where neurosecretory material is formed[1]; the following enzymes have been demonstrated histochemically: acetylcholinesterase[2], acid phosphatase[3], and succinic dehydrogenase[4]. The influence of chlorpromazine upon the organism is primarily due to its depressive effect upon the central nervous system in the formatio reticularis of the brain stem and in the hypothalamus of the diencephalon[5]. We have previously investigated the influence of chlorpromazine upon the neurosecretory substance of the hypothalamic magnocellular nuclei and upon the secretion of antidiuretic hormone, and we found that chlorpromazine depresses the hypothalamus[6]. The purpose of the present study is to determine to what extent chlorpromazine affects the histochemically demonstrable acetylcholinesterase, acid phosphatase, and succinic dehydrogenase activity in the supraoptic and paraventricular nuclei of the hypothalamus.

Chlorpromazine (Largactil, May & Baker) was given subcutaneously, 25 mg/kg body weight, to six adult female albino rats for ten days. The control group consisted of an equal number of animals. The subjects were killed by rapid decapitation 4 h after the last injection. From their hypothalamus the acetylcholinesterase was determined according to Koelle's modification[7], the acid phosphatase according to Eränkö[8], and the succinic dehydrogenase according to Seligman and Rutenburg[9].

[2] E. Lauda, *Lehrbuch der inneren Medizin*, vol. 3 (Springer, Wien 1951), p. 223.

[1] E. Scharrer, Exper. *10*, 261 (1954).

[2] V. C. Abrahams, G. B. Koelle, and P. Smart, J. Physiol. *139*, 137 (1957).

[3] O. Eränkö, Acta physiol. scand. *24*, 1 (1951).

[4] N. Shimizu and N. Morikawa, J. Histochem. Cytochem. *5*, 334 (1957).

[5] R. S. Courvoisier, J. Fournel, R. Ducrot, M. Koslky, and P. Koetschut, Arch. int. Pharmacodyn. *92*, 305 (1953). – H. E. Himwich and F. Rinaldi, *Brain Mechanism and Drug Action* (Springfield 1957).

[6] E. Kivalo, U. K. Rinne, and P. Marjanen, Ann. med. exp. biol. Fenn., in press (1958).

[7] G. B. Koelle, J. Pharmacol. *114*, 167 (1955).

[8] O. Eränkö, Ann. med. exp. biol. Fenn. *29*, 287 (1951).

[9] A. M. Seligman and A. M. Rutenburg, Science *113*, 317 (1951).